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Investigation Physiological and Biochemical Characteristics of Sorghum (*Sorghum bicolor* L., var. Kimia) Affected Zeolite Consumption under Water Deficit Situation

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ABSTRACT

Current study was carried out to assessment the effects of zeolite on seed yield, physiological and biochemical traits of sorghum in response to different irrigation regime according split plot arrangement based on complete randomized block design with three replications in Varamin region (Central of Iran). The main plots consisted of different irrigation regime at four levels [Normal irrigation (I_1), cut irrigation in vegetation stage (I_2), cut irrigation in flowering stage (I_3), cut irrigation in grain filling stage (I_4)]. Zeolite in three levels [nonuse zeolite or control (Z_1), 6 $t.ha^{-1}$ (Z_2) and 12 $t.ha^{-1}$ zeolite (Z_3)] belonged to subplots. Results of analysis of variance indicated that the interaction effects of different irrigation regime and zeolite application on all measured traits were significant at 1% probability level. According result of mean comparison of interaction effect of treatments the maximum of seed yield (4270.333 $kg.ha^{-1}$) was obtained for normal irrigation with use 12 $t.ha^{-1}$ zeolite and minimum of that (1601.000 $kg.ha^{-1}$) was for cut irrigation in grain filling stage with nonuse zeolite. Proline content increased as the amount of water deficiency and has a direct relationship with extinction coefficient, so the highest proline content and extinction coefficient were assigned of cut irrigation in vegetative stage with nonuse zeolite treatment. The maximum amount of HCN and Tannin (346.70 $mgr.gr^{-1}$, 48.867 $mg.gr^{-1}$) were achieved at cut irrigation in grain filling stage with nonuse zeolite. According to the obtained results it seems that assimilate has an effective role in the production of total biomass and seed yield in the period before and after flowering. The zeolite application 6 $t.ha^{-1}$ could be achieved acceptable LAI better than other treatments.

Keywords*:* *Extinction coefficient, Proline, Radiation use efficiency.*

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INTRODUCTION

Forage crops play an important role in supplying energy and protein to livestock (Eskandari *et al.*, 2009). In breeding of forage crops, increase of yield and forage quality are the main factors which play prominent role in the introduction of new varieties. Forages with good quality should have high dry matter yield, energy, digestibility and low fiber for optimal fermentation in the silo and storage. (Curran and Posch, 1999). Changes in climatic condition in recent decades caused reduction of amount of rainfall in arid and semi-arid regions in the world especially in Middle East. Due to shortage of water resources and sequential cropping in many areas, it is necessary to regulate the irrigation water, because this would cause inadequate irrigation (Alfi and Azizi, 2015). One of the most important factors that can limit crop production is the availability of water. Water is economic type of the foremost resources all around the world particularly in arid and semiarid areas (Selote *et al.,* 2004). Water is about to become increasingly limited for crop production (Martineau *et al.*, 2017). In arid and semi-arid areas of the world, water is the principal limiting factor of agricultural production primarily due to low and/or uneven distributions of annual rainfall (Keshavarz Afshar *et al.*, 2014). Water deficit stress causes an increase in solute concentration in the soil and root zone environment leading to an osmotic flow of water out of plant cells. This in turn causes the solute concentration inside plant cells to increase, thus lowering water potential and disrupting membranes along with essential processes like photosynthesis. These drought-stressed plants consequently exhibit poor growth and yield (Moser *et al.*, 2006). Drought is one of the factors which threaten agriculture products in most parts of the world (Abolhasani and Saeidi, 2004). It causes stress in plants and is not only caused by the reduction of rainfalls and great heat, but in the cases where there is moisture in the soil, this moisture cannot be used for plants for some reasons such as excessive soil salinity or soil frost, and plants will be stressed (Baydar and Erbas, 2005). Drought and water shortage are considered an objective reality. In the past, water crisis was not as significant as today, since the population was less, but with the population increase by about six times and the need for more food during the last 100 years, the incidence of this crisis has become more evident than the past (Chimenti *et al.*, 2002). Tolk *et al.* (2013) also reported that under drought conditions, the persistent green hybrid maintained yield by retaining greater seed numbers. In many regions of the world like Iran, drought stress is one of the most important factors that decrease agricultural crop production. Flowering, pollination and seed filling are sensitive stages to drought stress in plants (Thomas *et al.*, 2004). Improving the efficiency of water use in agriculture is associated with increasing the fraction of the available water resources that is transpired because of the unavoidable association between yield and water use (Jaleel *et al.*, 2007). So such materials can reduce losing soil moisture in arid and semi-arid regions by soil physical improvement. These storage tanks absorb water provided by irrigation and rainfall and reduced permeability of soil. In drought stress condition, water saved in the polymer is gradually depleted and reduces need for re-irrigation. According to result of Zamanian (2008), using zeolite can preserve soil moisture for a long time; consequently application of zeolite can decrease the effects of drought stress on crop plants. In regions where water scarcity is the principal limiting factor for cultivation, farmers are interested in using some methods to deduce injurious effects of water deficiency. One possible approach to reducing the effect of drought on plant productivity is through the addition of zeolite to soil (Manivannan *et al*., 2007). Cultivation of short season plants could be a suitable strategy for second cultivation in arid regions (Wang *et al*., 2003). However, some materials such as crop residuals, mulch plants, waste, litter, straw, stubble, and other synthetic materials like hydro plus zeolites could be used to save soil moisture (Silberbush *et al*., 1993). Zeolites are highly hydrophilic due to low cross-links in their structure (Huang and Petrovic, 1994). Zeolites may have great potential in restoration and reclamation of soil and storing water available for plant growth and production (Zhang *et al*., 2007). Chemical treatment and agronomical crop management practices have been tried to reduce the drought effects (Manivannan *et al*., 2007), but the application of zeolite to discharged plants attracted little attention. There are more than fifty known naturally occurring the zeolites (Coruh, 2008). Natural zeolites are hydrated aluminosilicates with comprising the silica and aluminum tetrahedral which result in stable three-dimensional framework. This honeycomb structure is generally very open, containing the channels and cavities, which are filled with the cations and water molecules (Karapinar, 2009). The cations are bound by weaker electrostatic bonds, increasing their mobility and the capability of being exchanged with cations present in the solution (Maranon *et al.,* 2006). Gholizadeh *et al.* (2010) showed that the increasing of zeolite and water stress have a significant effect on most of measured growth parameters. Zeolite provides an ideal physical root zone media for bent grass putting greens due to its particle size distribution, which provides a firm surface for foot traffic while remaining highly permeable (Huang and Petrovic, 1994). Zeolite is used as a soil additive, nutrient reservoir and super absorbent in soil. Application of some additives such as zeolite makes it possible to use infrequent rainfalls and limited water resources for preservation and storage of water in soil. Zeolites are micro porous, crystalline aluminosilicates of alkali and alkaline materials that have a high internal surface area (Silberbush *et al*., 1993). Zeolites are hydrated aluminosilicates of alkaline with open three-dimensional structure and are able to lose or gain water reversibly and exchange extra framework cations, both without crystal structure changes (Mumpton, 1999). Zeolites can act as water moderators and can absorb up to 55% water of their own weight, later on this water released slowly as per plant water demand (Pisarovic *et al*., 2003). Zeolite minerals are hydrated aluminosilicates of alkali or alkaline-earth metals, structured in a three dimensional rigid crystalline network, formed by tetrahedral AlO_4 and SiO_4, whose rings join in a system of canals, cavities and pores. These minerals are characterized by the ease of retaining and releasing water and exchanging cations without structural changes (Mumpton and Magica, 1999) and can potentially be used in field or substrate cultivation (Harland *et al.*, 1999). There are over 40 species of natural zeolites, of which clinoptilolite is apparently the most abundant, both in soils and in sediments (Ming and Dixon, 1987). Sorghum is the fifth most important cereal crop grown for human consumption in the world being surpassed only by rice, wheat, barley and corn. Most of sorghum grown in Asia and the African tropics is used for human food and also fed to livestock or poultry (Gul *et al.*, 2005). Sorghum is a drought resistant summer annual crop (Aishah *et al.*, 2011). Sorghum is a major cereal food crop in many parts of the world (Zebrini and Thomas, 2003), it is particularly important as a human food resources and folk medicine (Ryu *et al.*, 2006). Sorghum has high resistance to drought which makes it a suitable crop for semi-arid areas (Marsalis *et al.*, 2010), especially its higher productivity under dry conditions compared to corn (Tabosa *et al.*, 1986), because the sorghum needs lower irrigation requirements (Lamm *et al.*, 2007), and depletes less water from the soil than maize (Merrill *et al.*, 2007). Sorghum is grown as fodder crop due to the poor pollination and seed set during the extremely hot dry season (April-August) in the south provinces of Iran. Applying superabsorbent polymers can increase the water-holding capacity of soils and reduce the harmful effects of short-term drought in drought-prone arable areas (Karimi *et al.*, 2007). The objective of this study was to determine the effect of zeolite on sorghum yield under normal and drought stress conditions.

MATERIALS AND METHODS

Field and Treatments Information

Current study was carried out to assessment the effects of zeolite on seed yield, physiological and biochemical traits of sorghum (*Sorghum bicolor* var. Kimia) in response to different irrigation regime according split plot arrangement based on complete randomized block design with three replications in Varamin region (Central of Iran) during 2011. The main plots consisted of different irrigation regime at four levels [Normal irrigation (I_1), cut irrigation in vegetation stage (I_2), cut irrigation in flowering stage (I_3), cut irrigation in grain filling stage (I_4)]. Zeolite in three levels [nonuse zeolite or control (Z_1), 6 t.ha^{-1} (Z_2) and 12 t.ha^{-1} zeolite (Z_3)] belonged to subplots. The place of research is located at 19':35"N latitude, 39':51"E longitude, with an altitude of 1000m above sea level. This region has a semiarid climate (<200 mm annual rainfall). The soil was montmorillonite clay loam, low in terms of nitrogen (0.4 mg.kg^{-1}), low in terms of organic matter (7 mg.kg^{-1}), alkaline with a pH of 7.4 and EC was 0.87 ds.m^{-1}.

Farm Management

Initially, Phosphorus-fed nutrient was added to the plant by applying 100 kg.ha^{-1} triple superphosphate after cultivation. Nitrogen fertilizer was added in three periods; application of 50% N at cultivation time, application of 25% N fertilizer at stem elongation stage and application of 25% N fertilizer at beginning of flowering stage. A subplot size of 3.6×5 m was used, with six rows five meter long each. Uniformity of sowing depth was achieved by using a hand dibbler to make holes with 5-7 cm depth. The space between rows was 60 cm wide. All the experimental units were irrigated after planting.

Measured Traits

Seed yield was calculated in each split-plot after seed moisture reached 14% and the weight of each seed was determined after counting. The average of plant height between soil surface and plant head is considered to measure the plant height. For this purpose, 20 cm of stem is chosen randomly and measured according to cm. To measure chlorophyll content, five samples were taken for any treatment and sampling was performed when the leaves were green. A portable Minolta device was used to measure chlorophyll content and after averaging, the amount of chlorophyll was determined for any treatment. Calculation of the extinction coefficient (k): the daily light reached to canopy surface (I_o) was simulated by using latitude and the number of daylight hours through equations provided by Nassiri Mahalati and Kropft (1997) and in order to calculate the extinction coefficient (k) in the treatments (different plots), available light at the range 0.4 to 0.7 micromoles of photons per second was measured above and below the canopy on given 5 dates (coincident with the calculation of leaf area and dry matter) by using bar photometer (model sun scan ssl-VM-1.05) made in England (Delta-Devices T). The photometry was performed at a time range, from one hour prior to the afternoon to one hour later it. In order to measure the light of above canopy (base light), photometer was kept well balanced over the green bean plants and the amount of incoming radiation above the canopy was noted. Also photometry was performed to show the role of vegetation in light absorption in canopy on the ground surface and under the canopy. Then the extinction coefficient was calculated by excel software by using the beer lambert law through calculating the line slope of the regression with the natural logarithm of light transmission (I_i) to an incident light to the canopy level (I_o) versus **Equ. 1.** LAI: $I_i/I_o=e\text{-}^{kl}$. Where (I_o) denotes active photosynthetic radiation in upper part of the plant, (I_i): active photosynthetic radiation in i layer of leaves, k: extinction coefficient or reduction of radiation, e: base of natural logarithm that is equal to 2.71827 and l: leaf area index in *i* layer (Floyed and ShtonThomes, 2007). In order to calculate the radiation use efficiency (RUE), it requires calculation of the dry matter (DM) and the accumulative radiation (PAR_{absorbed}). Researches of Monteith (1997) showed that there is a kind of linear relationship between production of dry matter and the amount of absorbed radiation (PAR_{adsorbed}). The slope of this line that is called RUE is a criterion of net photosynthesis i.e. **Equ. 2.** DM= RUE×(PAR_{adsorbed}). In order to calculate the accumulative radiation, the model Intercom modified by Nassiri Mahalati and Kropfh (1997) was used. Thus, at first amount of the light penetrated into the canopy (I_o) (obtained from equations of Nassiri Mahalati and Kropfh (1997) was calculated by latitude and sunlight hours obtained from regional weather station and the light reached under shading (l_i) (calculated by the tubular photometer). Then the amount of light absorbed by the canopy during the growth period was calculated daily by considering the extinction coefficient (k) and leaf area index (LAI) and according to it, the accumulative radiation was obtained. The RUE was drawn by calculating the slope of the regression line among dry matter and absorbed accumulative radiation with Excel software (Monica *et al*., 2003). To measure LAI (leaf area index), after separating the leaves, the leaves area was measured immediately in the samples using the portable LAI meter, model IM-300. Measurement of total digestible nutrients (TDN) is done with using laboratory incubator methods (Ankom Daisy). Measurement of Tannin was done with using laboratory methods (Protein Precipitation) (Makkar *et al.,* 1988). To measured proline content leaf samples (0.2 gr) were homogenized in a mortar and pestle with 3 ml sulphosalicylic acid (3% w/v), and then the homogenate was centrifuged at 18,000g for 15 min. Two milliliters of the supernatant were then put into a test tube into which 2 milliliters of glacial acetic acid and 2 milliliters of freshly prepared acid in ninhydrin solution (1.25 g ninhydrin dissolved in 30 milliliters glacial acetic acid and 20 milliliters 6 molar ortho-phosphoric acid) were added. Tubes were incubated in a water bath for 1 hour at 100°C, and then allowed to cool up to room temperature. Four milliliters of toluene were added and mixed on a vortex mixer for 20 seconds. The test tubes were allowed to stand for at least 10 min to allow the 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 nanometer by spectrophotometer (GBC, Cintra 6, and Australia). The concentration of proline content was calculated by proline standard curve and was expressed in mili mol per gram of fresh weight (Bates *et al.*, 1973). Also hydrogen cyanide (HCN) was measured by the titration method (Williams, 1990).

Statistical Analysis

Data analyzed with MSTAT-C software. Duncan tests ($p< 0.05$) was applied for mean comparisons.

RESULTS AND DISCUSSION

Plant Height

According result of analysis of variance effect of different irrigation regime and zeolite on plant height was significant at 5% and 1% probability level, respectively also interaction effect of treatments was significant at 1% probability level (Table 1). Drought stress is reduced in plant during vegetative growth stage, but water deficit stress does not decrease plant growth and elongation after flowering stage. Mean comparison result of interaction effect of treatments revealed the highest plant height (119.3 cm) was achieved in the cut irrigation in grain filling stage and use 12 t.ha^{-1} zeolite and the lowest one (60.4 cm) belonged to cut irrigation in the vegetative stage and nonuse zeolite (Table 2). Alfi and Azizi (2015) reported using the zeolite leads to higher plant height in all three drought stress conditions.

Table 1. The ANOVA results of studied traits affected irrigation and zeolite

S.O.V	df	Plant height	Seed yield	Extinction coefficient	Radiation use efficiency
Replication	2	21.09^{ns}	45793.083^{ns}	0.005^{ns}	0.007*
Irrigation (I)	3	2289*	11977124.63**	0.033**	0.189**
Error I	6	5.333	47510.491	0.001	0.002
Zeolite (Z)	2	843.083**	1051166.083**	0.003^{ns}	0.001^{ns}
I*Z	6	154.306**	23215.713**	0.067**	0.009**
Error II	16	3.125	4961.472	0.010	0.002
CV (%)	-	8.86	12.43	6.45	4.17

** Means significant in 0.05 and 0.01 level of probability respectively and ^{ns}: non-significant

Continue Table 1.

S.O.V	df	Chlorophyll	Proline	TDN	HCN	Tannin
Replication	2	5.361^{ns}	0.003^{ns}	1.583^{ns}	36.950^{ns}	0.663^{ns}
Irrigation (I)	3	12.119**	0.197*	441.435**	84712.187**	919.332**
Error I	6	1.625	0.025	1.880	123.006	0.567
Zeolite (Z)	2	16.742**	0.111*	2.583*	67.848*	168.277**
I*Z	6	6.918**	0.905**	4.324**	2795.636**	17.054**
Error II	16	2.398	0.011	0.681	11.157	0.309
CV (%)	-	6.32	3.87	3.87	4.2	3.4

*, **Means significant in 0.05 and 0.01 level of probability respectively and ^{ns}: non-significant

Use of zeolite caused 5.62% and 3.33% improvement for plant height of corn genotypes in normal irrigation and severe drought stress respectively, in compare with non using zeolite.

Seed Yield

Result of analysis of variance revealed effect of different irrigation regime, zeolite and interaction effect of treatments on seed yield was significant at 1% probability level (Table 1). According result of mean comparison of interaction effect of treatments the maximum amount of seed yield trait (4270.333 kg.ha^{-1}) was obtained for normal irrigation with consumption 12 t.ha^{-1} zeolite and minimum of that (1601.000 kg.ha^{-1}) was for cut irrigation in grain filling stage with non consume zeolite (Table 2). Quanchang and Cheng (2008) expressed Zeolite consumption is increased seed yield; this research is consistent with Um *et al.* (1998) results. The results recorded in current study significantly increased seed yield when plants were irrigated completely.

Table 2. Means comparison effect of treatments on measured traits

Treatment		Plant height (cm)	Seed yield (kg.ha^{-1})	Extinction coefficient	Radiation use Efficiency (gr.µj.m^{-2})
I_1	Z_1	108^{*ab}	3839.000^{b}	0.489^{c}	2.076^{ab}
	Z_2	113.7^{ab}	4270.333^{ab}	0.463^{d}	2.367^{a}
	Z_3	115.4^{ab}	4287.667^{a}	0.468^{c}	2.273^{ab}
I_2	Z_1	60.4^{c}	3204.333^{c}	0.645^{a}	1.835^{b}
	Z_2	93.4^{b}	3880.667^{b}	0.609^{ab}	1.984^{ab}
	Z_3	93.33^{b}	3946.000^{ab}	0.611^{ab}	1.970^{ab}
I_3	Z_1	108^{ab}	1670.667^{e}	0.557^{abc}	1.915^{ab}
	Z_2	117.33^{ab}	2225.000^{d}	0.531^{bc}	1.993^{ab}
	Z_3	117.67^{ab}	2167.667^{d}	0.526^{bc}	1.963^{ab}
I_4	Z_1	109.7^{ab}	1601.000^{f}	0.549^{abc}	1.925^{ab}
	Z_2	118^{ab}	1995.667^{de}	0.530^{bc}	1.987^{ab}
	Z_3	119.3^{a}	1958.000^{de}	0.533^{bc}	1.986^{ab}

*Means with the same letter in each column have not statistically significant difference

I_1: Normal irrigation, **I_2**: Cut irrigation in vegetative stage, **I_3**: Cut irrigation in flowering stage, **I_4**: Cut irrigation in grain filling stage. **Z_1**: nonuse of zeolite, **Z_2**: 6 t.ha^{-1}, **Z_3**: 12 t.ha^{-1}

Continue Table 2.

Treatment		Chlorophyll (mg.gr^{-1})	Proline (µgr.gr^{-1})	TDN (%)	HCN (mg.gr^{-1})	Tannin (mg.gr^{-1})
I_1	Z_1	45.57^{*ab}	0.749^{c}	58.000^{ab}	98.633^{c}	23.333^{abc}
	Z_2	51.07^{a}	0.459^{e}	62.000^{a}	85.133^{d}	21.033^{c}
	Z_3	46.54^{ab}	0.509^{d}	60.000^{ab}	89.600^{c}	21.633^{bc}
I_2	Z_1	34.33^{c}	1.001^{a}	52.667^{ab}	280.600^{b}	28.967^{abc}
	Z_2	42.30^{ab}	0.856^{b}	57.000^{ab}	141.733^{c}	24.667^{abc}
	Z_3	40.96^{ab}	0.874^{ab}	57.000^{ab}	143.233^{c}	25.400^{abc}
I_3	Z_1	36.30^{b}	0.938^{ab}	43.667^{bc}	321.067^{ab}	46.900^{ab}
	Z_2	43.53^{ab}	0.797^{bc}	51.000^{b}	264.533^{b}	36.167^{abc}
	Z_3	43.00^{ab}	0.804^{bc}	50.333^{b}	271.867^{b}	37.033^{abc}
I_4	Z_1	37.70^{b}	0.892^{bc}	41.000^{c}	346.700^{a}	48.867^{a}
	Z_2	45.90^{ab}	0.766^{bc}	46.667^{bc}	272.667^{b}	38.300^{abc}
	Z_3	45.06^{ab}	0.771^{bc}	45.667^{bc}	279.867^{b}	40.600^{abc}

*Means with the same letter in each column have not statistically significant difference

I_1: Normal Irrigation, **I_2**: Cut irrigation in vegetative stage, **I_3**: Cut irrigation in flowering stage, **I_4**: Cut irrigation in grain filling stage. **Z_1**: nonuse of zeolite, **Z_2**: 6 t.ha^{-1}, **Z_3**: 12 t.ha^{-1}

It seems that Zeolite increases water retention capacity and thus water stress intensity will be decreased. Zeolite application was more effective under stress conditions than normal irrigation condition. Igbudun (2006) showed that the crop yield response was very much dependent on the amount of water consumed at different crop development stages than the overall seasonal water consumption. Therefore, cut irrigation in vegetative stage prevented the formation of favorite canopy and reduced length of panicle. Daneshian *et al.* (2006) announced that seed yield decreased in drought conditions, reducing the current photosynthesis at the during the seed filling period. But using the Zeolite increases nitrogen use efficiency (Roshdi *et al.*, 2005) plant was able to make the necessary amino acid in the water deficit that reduces the adverse effects. Alfi and Azizi (2015) reported using zeolite in all drought stress conditions including normal irrigation regime, mild and severe drought stress caused significant increase in maize forage yield. Also zeolite increased most of the quantitative traits in maize. Using zeolite as 10 ton.ha^{-1} caused 10.4% increasing in forage yield. Therefore, considering water shortage in drought area of the country and also importance of maize as a forage plant, application of zeolite can be useful to save more water that leads to produce more yields.

Extinction Coefficient

According result of analysis of variance effect of different irrigation regime and interaction effect of treatments on extinction coefficient was significant at 1% probability level but effect of zeolite was not significant (Table 1). Evaluation mean comparison result of interaction effect of treatments showed in different irrigation regime the maximum extinction coefficient (0.645) was noted for cut irrigation in vegetative stage with nonuse of zeolite and minimum of that (0.463) belonged to normal irrigation with use 6 t.ha^{-1} zeolite (Table 2).

Radiation Use Efficiency (RUE)

Radiation use efficiency can also be improved by increasing absorption of photosynthesis active radiation (PAR) by leaves as well as by improving the overall plant photosynthesis rate in stressful conditions. In addition to increasing the absorption of radiation during the growth period, improving the rate of dry matter production per unit of used radiation (i.e. RUE) by the crop is an important and practical measure in evaluating the production of biomass in the crop. Thus, dry matter production under normal conditions is a function of time, PAR, absorbed PAR and RUE (Tollennar and Aguilera, 1992). Monteith (1972) reported that RUE can be relatively constant. He has reported that RUE is about 1.4 g.MJ^{-1} for field crops. In contrast, other researchers have shown that RUE is variant for different cultivars (Jost and Cathren, 2000). Result of analysis of variance revealed effect of different irrigation regime and interaction effect of treatments on radiation use efficiency was significant at 1% probability level but effect of zeolite was not significant (Table 1). According result of mean comparison of interaction effect of treatments maximum of radiation use efficiency (2.367 gr.µj.m^{-2}) was obtained for normal irrigation with use 6 t.ha^{-1} zeolite and minimum of that (1.835 gr.µj.m^{-2}) was for cut irrigation in vegetative stage with nonuse of zeolite (Table 2).

Chlorophyll Content

Chlorophyll concentration has been known as an index for evaluation of source (Zobayed *et al.*, 2005), therefore decrease of this can be consideration as a no stomata limiting factor in the drought stress conditions. There are reports about decrease of chlorophyll in the drought stress conditions (Kuroda *et al*., 1990). Also, it is reported that chlorophyll content of resistant and sensitive cultivars to drought and thermal stress reduced. Chlorophyll and higher carotenoids with stress tolerance in plants is associated (Kraus *et al*., 1995) with the chlorophyll fluorescent measuring a relatively new technology that in recent years can study the effects of different stresses including drought, salinity and temperature on photosynthetic efficiency (or yield) of leaves in the farm (or field) and greenhouse conditions convention is used (Zobayed *et al*., 2005). Drought stress will reduce concentration of chlorophyll b more than chlorophyll a. For the first time, accumulation of proline in plant tissues that have missed water was reported in 1954 (Zobayed *et al*., 2005). The water synthesis of chlorophyll is very important, after a heavy rain the amount of chlorophyll increases, but in the arid time its value decreases. On the other hand, if the soil is water saturated, leaves chlorophyll content decreases. The amount of leaf water needed to maintain the maximum amount of chlorophyll should be high (Bohrani and Habili, 1992). In green plants chlorophyll tissue in leaves under environmental stress in susceptible cultivar is decreased but with an increased resistance. Leaves in the susceptible cultivar have a darker green color. Rapid loss of chlorophyll in cold-sensitive cultivars causes a decrease in photosynthetic activity. Several environmental factors cause chlorosis (yellowing) in plants. Chlorophyll is one of the basic pigments in plants, with its concentration reduction causing chlorosis, reduction in both growth and yield (Khosh and Ando, 1995). According result of analysis of variance effect of different irrigation regime, zeolite and interaction effect of treatments on chlorophyll content was significant at 1% probability level (Table 1). Assessment mean comparison result of interaction effect of treatments indicated the maximum chlorophyll content (51.07 $mg.gr^{-1}$) was noted for normal irrigation with use 6 $t.ha^{-1}$ zeolite and minimum of that (34.33 $mg.gr^{-1}$) belonged to cut irrigation in vegetative stage with nonuse of zeolite (Table 2). Chimenti *et al.* (2002) that express occurrence of water deficit stress has significant difference on seed yield at end of flowering stage, but zeolite consumption prevented severe reduction of chlorophyll and radiation use efficiency, and increase seed yield. Xiubin and Zhabin (2001) showed that zeolite application improved water retention capacity and cation exchange capacity in arable soils. The plant does not have potential to attract it, this matter decreases LAI, RUE, efficiency of photosynthesis and assimilation. Chlorophyll stability is known as the index of drought stress, and high stability index means high chlorophyll-content (Modhan *et al*., 2008). Alfi and Azizi (2015) reported application of zeolite caused significant increasing in leaf chlorophyll content; the highest chlorophyll content (30.6%) was obtained from using zeolite treatment and the lowest content (27.3%) was obtained from control treatment. Albert and Thornber (1977) have investigated the effects of water deficit stress on the content and order of Mesophilic chlorophylls and vascular pod in corn leaves and stated that the amount of leaf's chlorophyll reduces as a result of water deficit stress and this reduction is due to the lack between chlorophyll's lamla. Drought stress also reduced the uptake of essential elements and photosynthetic capacity (Kandil *et al.,* 2001) as well as increased synthesis of chlorophyll. The decrease in chlorophyll content under drought is a commonly observed phenomenon (Nikolaeva *et al.,* 2010). The decrease in chlorophyll under water stress might be due to reduced synthesis of the main chlorophyll pigment complexes encoded by the cab gene family (Allakhverdiev *et al.,* 2003), or to destruction of the pigment protein complexes which protect the photosynthetic apparatus, or to oxidative damage of chloroplast lipids and proteins (Lai *et al.,* 2007).

Proline

Plants accumulate some kindof organic and inorganic solutes in the cytosol to raise osmotic pressure and thereby maintain both turgor and the driving gradient for water uptake (Rhodes and Samaras, 1994). Among these solutes, proline is the most widely studied (Delauney and Verma, 1993). The beneficial roles of proline in conferring osmotolerance have been widely reported (Bajji *et al*., 2000). The maintenance of leaf turgor under water stress might be achieved through proline accumulation in cytoplasm improving water uptake from drying soil (Oraki *et al*., 2012), leading to leaf area expansion, increase in photosynthesis and assimilate supply for growth. Proline also protects membranes, macromolecules and sub-cellular organelles under dehydrating stress (Anjum *et al*., 2011) and might be also a part of the stress signaling influencing adaptive responses (Mafakheri *et al*., 2010). Proline concentration has been shown to be higher in stress-tolerant than in stress-sensitive plants (Anjum *et al*., 2011; Oraki *et al*., 2012). Result of analysis of variance revealed effect of different irrigation regime and zeolite on proline content was significant at 5% probability level, also interaction effect of treatments was significant at 1% probability level (Table 1). Evaluation mean comparison result of interaction effect of treatments indicated maximum proline content (1.001 µgr.gr^{-1}) was noted for cut irrigation in vegetative stage with nonuse of zeolite and lowest one (0.459 µgr.gr^{-1}) belonged to normal irrigation with use 6 t.ha^{-1} zeolite (Table 2). Free proline amino acid is accumulated upon leaf desiccation in leaves of many plant species (Barnett and Naylor, 1996). In sorghum, leaves of water-stressed field-grown plants also accumulated proline to a level several times greater than that in non-stressed plants (Ehrler, 2004). Free proline accumulation in water-stressed leaves has been speculated to constitute an attribute of drought resistance or drought hardiness (Levitt 2008).

Total Digestible Nutrients (TDN)

According result of analysis of variance effect of different irrigation regime and zeolite on TDN was significant at 1% and 5% probability level, respectively also interaction effect of treatments was 1% probability level (Table 1). Assessment mean comparison result of interaction effect of treatments indicated maximum TDN (62%) was noted for normal irrigation with use 6 t.ha^{-1} zeolite and lowest one (41%) belonged to cut irrigation in grain filling stage with nonuse of zeolite (Table 2).

Hydrogen Cyanide (HCN)

Result of analysis of variance indicated the effect of different irrigation regime and zeolite on HCN was significant at 1% and 5% probability level, respectively also interaction effect of treatments was 1% probability level (Table 1). Compare mean comparison result of interaction effect of treatments indicated maximum HCN (346.700 mg.gr^{-1}) was noted for cut irrigation in grain filling stage with nonuse of zeolite and lowest one (89.600 $mg.gr^{-1}$) belonged to normal irrigation with use 6 $t.ha^{-1}$ zeolite (Table 2).

Tannin

According result of analysis of variance effect of different irrigation regime, zeolite and interaction effect of treatments on tannin was significant at 1% probability level (Table 1). Mean comparison result of interaction effect of treatments showed maximum tannin (48.867 $mg.gr^{-1}$) was noted for cut irrigation in grain filling stage with nonuse of zeolite and lowest one (21.033 $mg.gr^{-1}$) belonged to normal irrigation with use 6 $t.ha^{-1}$ zeolite (Table 2).

Leaf area index (LAI)

LAI indicates the amount of photosynthesis. When LAI decreases according to various factors, assimilate rate is reduced, so the amount of yield decreases and in this study it was evident that it corresponds to result of researches by Chaillou *et al*. (2003). Fig. 1 shows the changes in LAI at different levels of irrigation. The Normal and cut irrigation in flowering and cut irrigation in seed field treatments with water availability in the upper layers of the soil where the root system normally improve thus these treatments could obtain better LAI. The plant could make more desirable spatial position and array and with timely root development to use soil nitrogen better and with aerial components development to enjoy LAI better. In cut irrigation in Vegetative treatment, this rate decreased sharply. Even in the later growth stages could not compensate for this defect. But results showed in this treatment that all leaves to absorb sunlight, yield drop are less than other cut irrigation treatments. But in the third treatment cut-irrigation when the flowering occurred while the plant lost some of leaves to compensate tensions.

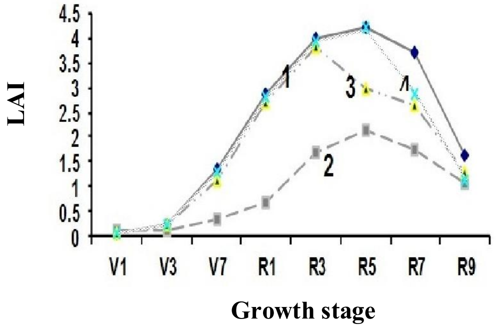

Fig. 1. Variation of LAI in response of different irrigation regime. **1**= Normal irrigation, **2**= cut irrigation in vegetation stage, **3**= cut irrigation in flowering stage, **4**= cut irrigation in grain filling stage.

Also leaf development process was stopped in order to keep the plant under water deficit stress conditions. This cause was reduced in this treatment LAI relatively. Fig. 2 shows the changes in LAI in treatments of Zeolite. In flowering stage, the maximum LAI was found to produce photosynthetic materials. Then making leaves is stopped and gradually lower leaves began to yellow and thus LAI is reduced. The rise of third leaves, LAI is closer together in different treatments, and then zeolite use is obvious to their treatments.

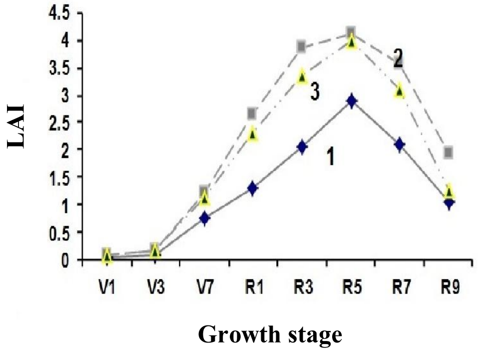

Fig. 2. Variation of LAI in response of different amount of zeolite. **1**= nonuse zeolite or control, **2**= use 6 $t.ha^{-1}$ zeolite, **3**= use 12 $t.ha^{-1}$ zeolite.

This process coincided exactly with the necessary maximum photosynthetic material in order to increase growth, for increase production. The Zeolite application 6 $t.ha^{-1}$ could be achieved by LAI better than other treatments. That treatment, plant was access to water and nutrient of photosynthetic better, for the production. Generally, Leaf senescence occurs because of the source–sink (in terms of physiological hunger) is low, or because of being higher than the source–sink (Excessive accumulation of assimilates).

CONCLUSION

The highest extinction coefficient and proline were obtained under cut irrigation in vegetative stage with nonuse zeolite and the lowest radiation use efficiency and chlorophyll content allocated to the same treatment. It seems the water deficit stress has a negative effect in the early stages of the formation of the leaves, and led to the shrinking of the leaf area and unavailability of the zeolite to reduce ion exchange capacity of the soil. Also the highest radiation use efficiency and chlorophyll were obtained in normal irrigation with zeolite 6 $t.ha^{-1}$. TDN content seed was inversely related to the amount of HCN, tannin and proline. The lowest proline, HCN and Tannin were assigned to normal irrigation and 6 $t.ha^{-1}$ zeolite treatments and same treatment was obtained from the most amount of TDN (62%). Also the maximum amounts of HCN and Tannin were achieved at cut Irrigation in grain filling stage with nonuse zeolite. According to the obtained results it seems that assimilate has an effective role in the production of total biomass and seed yield in the period before and after flowering. The zeolite application 6 $t.ha^{-1}$ could be achieved acceptable LAI better than other treatments.

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