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Zeolite Use Efficiency Variation under Water Deficit Stress in Grass Pea and Lentil

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To evaluate the effect of different application rates of zeolites under drought stress conditions on the protein and biomass production, and zeolite use efficiency in grass pea (Lathyrus sativus L.) and lentil (Lens culinaris L.), two-way ANOVA based on randomized complete block design was conducted at Research Farm of Urmia University in 2012. Two levels of irrigation (irrigation at field capacity (FC) and 50 % FC) and four levels of zeolites (0, 10, 20 and 30 tons/ha) were applied. Results of ANOVA showed the significant effect of zeolite application on protein yield, protein harvest index, and zeolite use efficiency for protein and biomass production and significant effect of irrigation regime on protein yield, protein harvest index and zeolite use efficiency for grass pea protein production. There were significant effects of zeolite application on the biomass, protein yield, and protein harvest index, and significant effects of irrigation regime on protein yield and protein harvest index of lentil. However significant interaction effect between zeolite and irrigation on zeolite use efficiency for protein and biomass production in lentil was obtained. The results indicated that water deficit stress significantly decreased these traits, whereas the application of zeolite compensated the negative effect of the drought stress. Zeolite use efficiency for grass pea and lentil biomass and protein production decreased in high rates of mineral application (30 tons/ha). The highest protein yield (307.12 kg/ha) and protein harvest index (14.2 %) of grass pea, and protein yield (222.59 kg/ha), biological yield (2587.6 kg/ha), and protein harvest index (8.6 %) of lentil were obtained when 30 tons/ha of zeolite were applied. These findings strongly suggested that the irrigation intervals of grass pea and lentil could be extended by application of zeolite.

Keywords: Lathyrus sativus L., Lens culinaris L., water stress, yield, zeolite.

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Изменение эффективности применения цеолита при выращивании чины посевной и чечевицы в условиях водного стресса

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Для оценки влияния внесения в почву различных доз цеолитов на продукцию белка и биомассы в стрессовых условиях засухи, а также эффективности использования цеолитов при выращивании чины посевной (Lathyrus sativus L.) и чечевицы (Lens culinaris L.), на исследовательской ферме университета г. Урмия в 2012 году был проведен двухфакторный дисперсионный анализ (ANOVA) на основе полностью рандомизированного блочного плана. Были использованы два уровня орошения (полевая влагоемкость и 50% от полевой влагоемкости) и четыре режима внесения цеолитов (0, 10, 20 и 30 т/га). Результаты дисперсионного анализа показали достоверное влияние применения цеолитов на выход белка, содержание белка в единице сухой надземной массы (СБ) и эффективность применения цеолитов для повышения продукции белка и биомассы, а также достоверное влияние режима орошения на выход белка, СБ и эффективность использования цеолитов для повышения продукции белка чиной полевой. Отмечено достоверное влияние применения иеолитов на биомассу, выход белка и СБ, а также достоверное воздействие режима орошения на выход белка и СБ чечевицы. Однако также наблюдалось достоверное совместное влияние применения цеолитов и орошения на эффективность использования цеолитов для повышения продукции белка и биомассы чечевицей. Показано, что при воздействии водного стресса эти показатели достоверно снижались, в то время как применение цеолитов компенсировало негативный эффект засухи. Эффективность использования цеолитов для повышения продукции биомассы и белка чиной полевой и чечевицей снижалась при высокой дозе внесения минералов (30 т/га). Самый высокий выход белка (307,12 кг/га) и СБ (14,2 %) чины полевой, выход белка (222,59 кг/га), биологический урожай (2 587,6 кг/га), а также СБ (8,6 %) чечевицы были получены при внесении 30 т/га цеолитов. Эти данные убедительно свидетельствуют, что интервалы орошения чины полевой и чечевицы могут быть увеличены при внесении в почву цеолитов.

Ключевые слова: Lathyrus sativus L., Lens culinaris L., водный дефицит, урожай, цеолиты.

Introduction

Legumes (Fabaceae) are protein rich plants (Van Ek et al., 1997). Grass pea (*Lathyrus sativus* L.) contains 20-32 % protein (Ramachandran et al., 2005) and lentil (*Lens culinaris* L.) is one of the major legume crops all over the world including Iran. It is a cheap source

of high quality protein in diets of millions people in developing countries (Karadavut & Palta, 2010).

Water availability is one of the most limiting environmental factors affecting crop productivity and it is a well-known fact that crop growth is frequently subjected to water stress during its lifetime. Water stress drastically affects crop growth, ultimately leading to a massive yield and quality loss (Govindarajan et al., 1996; Habibzadeh et al., 2013; Hudak & Patterson, 1996; Faver et al., 1996). Drought stress is one of the most important abiotic stress factors which are generally accompanied by heat stress in dry season (Dash & Mohanty, 2001). In recent years, it has been shown that proper supplemental irrigation can increase crop yield by significantly improving soil moisture conditions and efficiency of water use by the crop (Deng et al., 2002). 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 vield and water use (Jaleel et al., 2007). Due to water deficits, the crop physiology is disturbed which causes a large number of changes in morphology and anatomy of plants.

Natural zeolites hydrated are aluminosilicates consisting of a stable threedimensional framework of silica and aluminum tetrahedra. This honeycomb structure is generally very open, containing channels and cavities, which are filled with cations and water molecules (Karapinar, 2009). Zeolite is a group of natural minerals with physical and physicochemical properties that can be utilized in various areas such as construction and agriculture. They are capable of absorbing part of the excess nutrients and also water, resulting in more balanced macronutrient cation ratios in the root environment and can keep water in root zone (Savvas et al., 2004). These results suggest that soil improvement with zeolites is important in plant management for sustaining a high yield production under water deficit conditions. Moser et al. (2006) reported that biomass was reduced by moisture stress and stated that yield was strongly related to biomass accumulated especially after flowering stage. Biomass was also reduced by water deficit. Zeolite application had desirable effects on protein content in medicinal pumpkin under all used irrigation regimes, so that, it decreased the adverse drought stress effects and caused to prevent loss of protein content in drought stressed plants by 23.01 % (Eskandari Zanjani et al., 2012).

The main purpose of this study is to evaluate reducing biomass production in *Lathyrus sativus* and *Lens culinaris* under water deficit stress, and compensate of this damage by zeolite usage. Furthermore, we will find the optimum amounts of zeolite and determine zeolite use efficiency for enhancing protein production in these two plants.

Materials and Methods

The study was conducted as a factorial experiment based on randomized complete block design (RCBD) with 8 replications. The experiment was carried out at the Agricultural Research Farm of Urmia University, Iran (37.53°N, 45.08°E, 1320 m above sea level) in 2012. The physical and chemical properties of soil are listed in Table 1.

Irrigation treatments including irrigation at field capacity (FC) and 50 % of FC were considered as the first factor (Table 2). Application of drought stress was initiated at two-leaf stage. Four levels of zeolite including

Table 1. Basic physical and chemical properties of the experimental field soil

Soil depth (cm)	Soil texture	Silt-Clay-Sand (%)	Organic carbon (%)	N (%)	P (mg/kg)	K (mg/kg)	EC×10³	рН
0-30	Loam	35-39-26	0.6	6	10.4	250	1.1	8.2

Date	29-Aug	31-Aug	2-Sep	4-Sep	7-Sep	11-Sep	14-Sep	18-Sep
Evaporation	8.1	6.2	7.9	6.2	8.5	7	4.9	4.8
FC	V	V	√	V	V	1	√	√
50 % FC		$\sqrt{}$		$\sqrt{}$		$\sqrt{}$		$\sqrt{}$

Table 2. Irrigation schedule used in the experiment (FC – irrigation at field capacity)

Table 3. Chemical and mineralogical composition of natural Western Azerbaijan zeolite (XRD analysis)

Parameter	%
SiO ₂	67.5
Al_2O_3	12.5
Na_2O	3.1
K_2O	4.4
CaO	1.6
Fe_2O_3	0.2-0.9
Loss on ignition	10-13
Major mineral: Clinoptololite-Quartz- Cristobalite	
Minor mineral: Calesite- Montmonlonite	

0, 10, 20 and 30 tons/ha were considered as the second factor. Zeolites were distributed in the soil layer 0-30 cm before sowing (Table 3). Plots were 100 cm long and 50 cm wide. The grass pea and lentil seeds were disinfected and sown on 31 July in five lines (10 cm inter and 5 cm intra row space). The plots were at enough distance from each other that triggered no competition for light absorption. Crops were harvested on 23^{rd} September.

To evaluate leaf dry weight, samples (leaves) were dried in an oven at 70°C and weighted. Leaf nitrogen content was determined by the micro-Kjeldahl method (Jackson et al., 1973). About 25 mg of samples were transferred to a micro-digestion tube and digested with 1 mL of low nitrogen concentrated H₂SO₄ and a few mg of 3:1 CuSO₄:K₂SO₄ mixture (Stuart, 1936). Leaf

protein content was calculated by multiplying total nitrogen content with factor 6.25 (Shahidi et al., 2001). Protein yield (PY) was calculated using the equation: PY= leaf biomass (kg/ha dw) × leaf protein (%). To determine biological yield, above-ground biomass (included leaves and stems) was harvested, dried at 75 °C, and then weighted. Harvest index was calculated as protein yield divided by biological yield. Zeolite use efficiency (ZUE) was defined as yield of plant product (kg/ha biomass or protein) per unit of used zeolite (kg/ha of zeolite):

ZUE for biomass=biological yield/zeolite per unit area of obtained biomass

ZUE for protein=protein yield/zeolite per unit area of obtained protein

Data analysis was carried out using SAS 9.1 software. Student-Newman-Keul's test (SNK) at $P \le 0.05$ was used to test for the significance of a difference between means.

Results

Lathyrus sativus

Results of ANOVA showed significant effect of irrigation on protein yield, protein harvest index and zeolite use efficiency for grass pea protein production. Significant effects of zeolite application on protein yield, protein harvest index and zeolite use efficiency for protein and biomass production of grass pea were obtained (Table 4).

The minimum value of leaf protein yield of grass pea (107.1 kg/ha) belonged to control treatment and did not differ significantly from

Table 4. Results of two-way ANOVA testing the effects of different irrigation regimes and zeolite application rates on *Lathyrus sativus* physiological traits

	,	MS						
Variation source	df	Biological yield	Protein yield	Harvest index of protein	ZUE for biomass	ZUE for protein		
Replication	2	0.0602 ^{ns}	0.0921 ^{ns}	2.3403 ^{ns}	0.0007 ^{ns}	0.00002 ^{ns}		
Zeolite	3	$0.1457^{\rm ns}$	0.2657**	33.7071**	0.0101**	0.00007^{**}		
Irrigation	1	0.0395^{ns}	0.2060^{*}	41.6646**	0.0017^{ns}	0.00009^*		
Zeolite×Irrigation	3	0.0181^{ns}	0.0179^{ns}	1.7723 ^{ns}	0.0009^{ns}	$0.000007^{\rm ns}$		
Error	14	0.0476	0.0418	2.0276	0.0006	0.000009		

[&]quot;ns": non-significant, *: significant at P < 0.05, **: significant at $P \le 0.01$, df: degree of freedom.

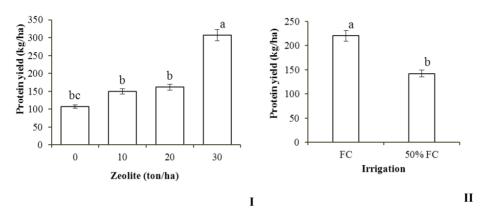


Fig. 1. Protein yield of *Lathyrus sativus* grown in soil treated with different amounts of zeolite (I) under various irrigation regimes (II). Bars labeled with the same letter are not significantly different at $P \le 0.05$ after SNK test

values obtained in the 10 and 20 tons/ha zeolite treatment. However, the higher amounts of zeolite caused significant increase in this trait. The maximum leaf protein yield (307.12 kg/ha) was obtained in the 30 tons/ha zeolite treatment (Fig. 1-I). In irrigation treatments, a maximum value of protein yield was obtained under normal irrigation regime whereas water stress significantly decreased protein concentration in plant leaves (Fig. 1–II). The maximum (12.93 %) and minimum (10.3 %) harvest index of grass pea protein were obtained under irrigation at 100 and 50 % of field capacity, respectively (Fig. 2-II). Application of zeolite significantly increased harvest index of protein. The minimum harvest index of grass pea protein (8.7 %) belonged to

control treatment, but the higher amounts of zeolite caused significant increase of this trait. The maximum harvest index of grass pea protein (14.2 %) was observed in the 30 tons/ha zeolite treatment (Fig. 2-I).

Comparison of means between variants with different levels of zeolite application indicated that the highest amount of ZUE for grass pea biomass production (0.138) was obtained when zeolite was applied in the amount of 10 tons/ha. On the other hand, the lowest value of ZUE for grass pea biomass production (0.62) was found at the highest dosage of zeolite (Fig. 3). The maximum zeolite use efficiency for protein (0.014) was obtained in the 10 tons/ha zeolite treatment, but it was reduced at higher

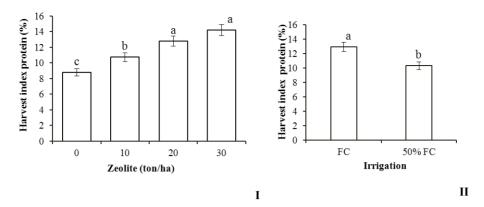


Fig. 2. Protein harvest index of *Lathyrus sativus* grown in soil treated with different amounts of zeolite (I) under various irrigation regimes (II). Bars labeled with the same letter are not significantly different at $P \le 0.05$ after SNK test

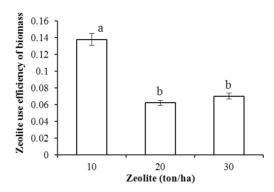


Fig. 3. Zeolite use efficiency for biomass production by *Lathyrus sativus* grown in soil treated with different amounts of zeolite. Bars labeled with the same letter are not significantly different at $P \le 0.05$ after SNK test

amounts of zeolite. So, the minimum value of zeolite use efficiency for grass pea protein production (0.0085) belonged to the 20 tons/ha zeolite treatment and did not differ significantly from the value obtained at 30 tons/ha (Fig. 4–I). These trends for zeolite use efficiency were due to decreasing protein content and yield under drought stress condition (Fig. 1 and 2).

There was positive significant correlation between biological and protein yield (r^2 =0.89), ZUE for biomass production and ZUE for protein (r^2 =0.91), protein harvest index and protein yield (r^2 =0.74), and protein harvest index and biological yield (r^2 =0.41) of *Lathyrus sativus* (Table 5).

Lens culinaris

Biological yield, protein yield, protein harvest index and zeolite use efficiency for lentil protein production were affected by zeolite application. There were also significant effects of irrigation on protein yield, protein harvest index and ZUE for protein production, and interaction between zeolite and irrigation on ZUE for biomass and protein production (Table 6).

The lowest biological yield of lentil (2131.6 kg/ha) was observed when 10 tons/ha of zeolite were applied and the highest value (2587.6 kg/ha) was observed in the 30 tons/ha zeolite treatment (Fig. 5).

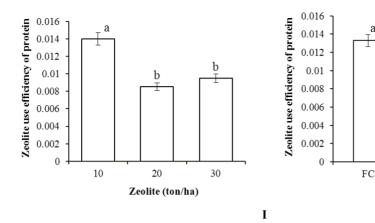


Fig. 4. Zeolite use efficiency for protein production in *Lathyrus sativus* grown in soil treated with different amounts of zeolite (I) under various irrigation regimes (II). Bars labeled with the same letter are not significantly different at $P \le 0.05$ after SNK test

Table 5. Correlation coefficients between analyzed traits of Lathyrus sativus

	Biological yield	Protein harvest index	ZUE for protein production	ZUE for biomass production
Protein yield	0.89**	0.74**	0.43ns	0.11 ^{ns}
Biological yield		0.41*	0.46^{ns}	$0.30^{\rm ns}$
Protein harvest index			$0.04^{\rm ns}$	-0.35^{ns}
ZUE for protein production				0.91**

[&]quot;ns": non-significant, *: significant at P<0.05, **: significant at P≤0.01.

The lowest leaf protein yield of lentil (146.57 kg/ha) was obtained in the control (without applying of zeolite) and the highest leaf protein yield (222.59 kg/ha) was obtained by applying of zeolite in amount of 30 tons/ha (Fig. 6–I). Drought stress significantly decreased protein yield in leaves. The maximum yield of protein was obtained under normal irrigation whereas water stress (irrigation at 50 % FC) significantly decreased protein concentration in plant leaves (Fig. 6-II).

The highest protein harvest index of lentil (8.6%) was obtained under irrigation at 100% of field capacity, and the lowest protein harvest index (6.6%) was observed under irrigation at 50% of field capacity (Fig. 7-II). Application of zeolite increased the protein harvest index. Whereas

the lowest protein harvest index of lentil (6.6 %) belonged to control treatment, higher amounts of zeolite significantly increased harvest index and the highest protein harvest index of *L. culinaris* (8.6 %) was obtained by applying 30 tons/ha zeolite (Fig. 7-I).

50% FC

II

Irrigation

Considering the results of analysis of variance (Table 6), comparison of means between different amounts of zeolite application showed that the highest amounts of ZUE for biomass production of lentil plants (0.21) was obtained by applying 10 tons/ha zeolite and irrigation at field capacity (Fig. 8). The increase of zeolite amounts caused ZUE reduction. The lowest ZUE (0.084) was found at the highest zeolite amounts (30 tons/ha) under irrigation both at field capacity and 50 % FC (Fig. 8).

Table 6. Results of two-way ANOVA testing the effects of different irrigation regimes and zeolite application rates on physiological traits of *Lens culinaris*

		MS						
Variation source	df	Biological yield	Protein yield	Protein harvest index	ZUE for biomass	ZUE for protein		
Replication	2	37871.312 ^{ns}	484.8401 ^{ns}	0.1569 ^{ns}	0.000006 ^{ns}	0.0000007^{ns}		
Zeolite	3	338516.635*	7447.5455**	4.0887**	0.0252^{ns}	0.0001^{**}		
Irrigation	1	$21266.594^{\rm ns}$	16464.2920**	35.4962**	0.00004^{ns}	0.00004**		
Zeolite×Irrigation	3	50116.216 ^{ns}	$462.5094^{\rm ns}$	0.2080^{ns}	0.0002^*	0.00001**		
Error	14	68058.923	665.5373	0.1561	0.00005	0.0000006		

[&]quot;ns": non-significant, *: significant at P < 0.05, **: significant at $P \le 0.01$, df: degree of freedom.

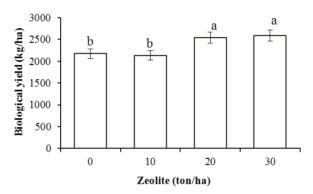


Fig. 5. Biological yield of *Lens culinaris* grown in soil treated with different amounts of zeolite. Bars labeled with the same letter are not significantly different at $P \le 0.05$ after SNK test

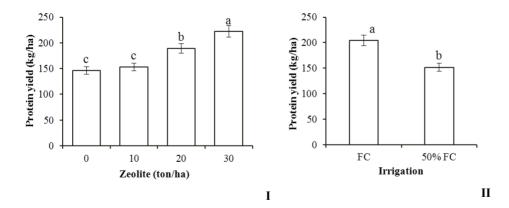


Fig. 6. Protein yield of *Lens culinaris* grown in soil treated with different amounts of zeolite (I) under various irrigation regimes (II). Bars labeled with the same letter are not significantly different at $P \le 0.05$ after SNK test

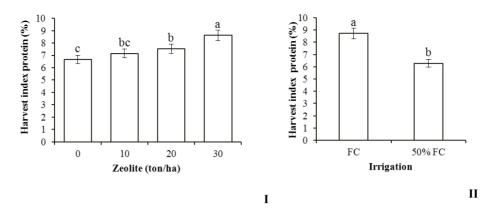


Fig. 7. Protein harvest index of *Lens culinaris* grown in soil treated with different amounts of zeolite (I) under various irrigation regimes (II). Bars labeled with the same letter are not significantly different at $P \le 0.05$ after SNK test

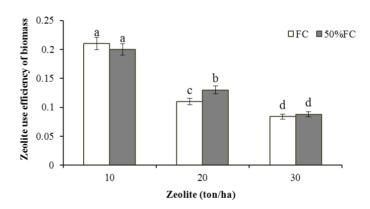


Fig. 8. Zeolite use efficiency for biomass production by *Lens culinaris* grown in soil treated with different amounts of zeolite under various irrigation regimes. Bars labeled with the same letter are not significantly different at $P \le 0.05$ after SNK test

Under both irrigation regimes, the highest ZUE for protein production of lentil was obtained by applying 10 tons/ha zeolite and it was higher under irrigation at FC for all zeolite treatments. The maximum ZUE for protein production (0.01846) belonged to normal irrigation regime and the 10 tons/ha zeolite treatment, and the minimum ZUE protein production (0.00646) was obtained under irrigation regime at 50 % FC by applying 30 tons/ha zeolite (Fig. 9).

ZUE for biomass production showed negative correlation with both protein yield (r²=-0.63) and biomass production (r²=-0.64). Zeolite use efficiency for protein production had negative

correlation with biological yield (r^2 =-0.57). However, there were significant positive correlation between protein and biological yield (r^2 =0.69), protein yield and protein harvest index (r^2 =0.88), and zeolite use efficiency for protein and biomass production (r^2 =0.86) in lentil (Table 7).

Discussion

The above results clearly indicated that any decrease in the amount of irrigation reduces traits of *Lens culinaris* and *Lathyrus sativus*, but the irrigation intervals for these plants could be extended by application of zeolite without great reduction of plant productivity. The maximum

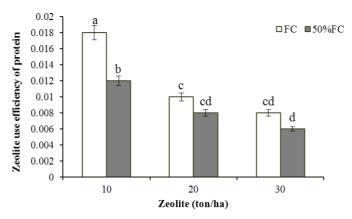


Fig. 9. Zeolite use efficiency for protein production in *Lens culinaris* grown in soil treated with different amounts of zeolite under various irrigation regimes. Bars labeled with the same letter are not significantly different at $P \le 0.05$ after SNK test

Table 7. Correlation coefficients between analyzed traits of Lens culinaris

	Biological yield	Protein harvest index	ZUE for protein production	ZUE for biomass production
Protein yield	0.69**	0.88**	-0.23ns	-0.63*
Biological yield		0.28^{ns}	-0.57*	-0.64*
Protein harvest index			$0.07^{\rm ns}$	-0.39 ^{ns}
ZUE for protein production				0.86**

[&]quot;ns": non-significant, *: significant at P < 0.05, **: significant at $P \le 0.01$.

plant protein content and yield, as well as zeolite use efficiency for protein and biomass production were observed under irrigation at 100 % field capacity.

Drought-induced decrease in total protein percent in safflower (*Carthamus mareoticus* L.) has also been reported (Abdel-Nasser & Abdel-Aal, 2002). The highest protein content in medicinal pumpkin was obtained after application of zeolite under normal irrigation (without disruption) and the lowest one was obtained when zeolite was not applied and irrigation was withheld at the fruit formation stage (Eskandari Zanjani et al., 2012). The decrease in plant protein contents might be due to increased photolytic activity. Proteins are hydrolyzed by proteases to release amino acids for storage and/or transport and for osmotic adjustment

(e.g. proline) during drought stress in plant. Osmotic adjustment, cellular macromolecules production, storage form of nitrogen, cellular pH maintenance, cells detoxification and free radicals scavenging are proposed functions of free amino acid accumulation (Parida et al., 2007). The accumulation of dehydrin-like proteins was detected in the roots and leaves of drought-stressed maize plants, which could protect plants from further dehydration damage (Mohammadkhani & Heidari, 2008). The changes in total soluble proteins under drought stress were in accordance with the findings Riccardi et al. (1998) and Ti-da et al. (2006) in maize.

Stone et al. (2001) stated that the yield was related strongly to biomass, especially which accumulated after flowering stage. Moser et al. (2006) also reported that biomass was reduced by

moisture stress. Chimenti et al. (2002) reported that despite significant effect of water deficit stress on total above ground biomass reduction at the end of flowering stage, zeolite consumption prevented severe reduction in biomass and harvest index.

Protein yield, biological yield and protein harvest index did not differ significantly in control and in the 10 tons/ha zeolite treatment, but increasing of the amount of applied zeolite had significant effect on these traits. Reducing of zeolite amount also increased zeolite use efficiency for biomass and protein in lentil and grass pea plants. Koljajic et al. (2003) reported that the increase of the amount of zeolite significantly increased the dry matter, protein and crude fiber contents in fresh beet pulp. Zeolite application had positive and enhancing effects on protein production in all irrigation treatments decreasing the adverse drought stress damages. It has been reported that mixtures of zeolite with other substrates increased plant yield in many species such as gerbera (Issa et al., 2001), cucumber (Gül et al., 2007), tomato (Al-Ajmi et al., 2009). Zeolite in substrates mixtures may promote anion and cation exchange capacity (Issa et al., 2001). Mixtures of zeolite and fertilizers also had positive effects on lettuce (Gül et al., 2005) and tomato yields (Valente et al., 1982).

In conclusion, it was observed that, under drought stress conditions, *Lathyrus sativus* and *Lens culinaris* demonstrated the lowest protein and biological yield, protein harvest index, and zeolite use efficiency for biomass and protein production. Zeolite application in regions which are exposed to late season drought stress can keep soil water content and improve plant growth and production. In general, zeolites as soil amendments improve water retention capacity, soil cation exchangeable capacity which leads to higher yield under drought stress conditions. Both zeolite use efficiency for biomass and for protein production were lowered with higher amount of applied zeolite.

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