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Influence of Zeolite and Biological Fertilizer under Different Irrigation Regime on Quantitative and Qualitative Traits of Sugar beet (*Beta Vulgaris* L.)

Tahereh Hasanabadi[*1] Davood Habibi[2] Hamideh Khalaj[3]

1- Young Researchers and Elites Club, Tehran North Branch, Islamic Azad University, Tehran, Iran.
2- Department of Agronomy and Plant Breeding, Karaj Branch, Islamic Azad University, Karaj, Iran.
3- Department of Agriculture, Payame-Noor University, P.O. Box: 19395-3697, Tehran, Iran.

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ABSTRACT

In order to study the effect of zeolite and biologic fertilizers application under different irrigation regime on yield and quantitative traits in sugar beet, a research project was conducted according split-split plot experiment based on randomized complete block design with four replicates. The main factor included irrigation regime at two levels (Normal and stress), sub factor included zeolite application at two levels (with and without application) and biological fertilizers at four levels [1- Non application of mycorrhiza, 2- Application of mycorrhiza 3- Non -inoculation of bacteria 4- Inoculation of bacteria (*Pseudomonas, Azotobacter, Azospirillum*)] belonged to sub-sub factor. Analysis of variance results showed that interaction effect of zeolite and mycorrhiza and non-application of bacteria under normal irrigation regime on all measured traits (Instead potassium and nitrogen content) was significant. Mean comparison of treatments indicated that $NZ_1M_1B_0$ treatment (Zeolite and mycorrhiza application and non-inoculation of bacteria under normal irrigation regime) had highest amount of root yield (73340 $kg.ha^{-1}$), white sugar content (11.88%) and white sugar content (16.47%) but treatments of NM_1B_0 (Mycorrhiza application and non-inoculation of bacteria under normal irrigation regime), DM_0B_0 (Non-application of mycorrhiza and bacteria under stress irrigation regime) and $DZ_0M_0B_0$ (Non-application of zeolite, mycorrhiza and bacterial under stress irrigation regime) had highest amount of potassium content (4.45 $meq.100g^{-1}$ sugar), amino-nitrogen (2.09 $meq.100g^{-1}$ sugar) and sodium content (11.95 $meq.100g^{-1}$ sugar), respectively. According to results of this research mycorrhiza inoculation and use of zeolite under water deficient conditions, caused improving sugar yield and consequently decreasing negative elements (Na, K, and N) under drought stress condition.

Keywords*:* *Bacteria, Nitrogen, Root yield, Sugar content.*

[*]**Corresponding Author**: Tahereh Hasanabadi ✉ t.hasanabadi@gmail.com

INTRODUCTION

Sugar beet generally considered as a crop of temperate region, and is spreading to subtropical countries where it can be grown successfully during winter season. It is having growth period of about half of sugarcane but productivity per unit time is higher and requires less water than sugarcane (Singh Brar *et al*., 2015). Sugar beet production is an essential component of agricultural economics in many countries. Development of sugar beet cultivars tolerant to drought stress is an important Method to prevent yield loss in dry conditions. In arid or semi-arid regions which plants faces water shortage, sugar beet yield loss may reach to more than 20% (Ober, 2001). Water and Fertilization are limiting factors for sugar beet production. Thus, they are favorable to choose the optimum rate and times of water and application from macro and micro nutrients to give the maximum yield and quality for sugar beet crop. Due to the shortage of water over the world, providing strategies such as proper irrigation methods, irrigation management, while offering ways to reduce and control the negative effects of water stress in plants and varieties more resistant to water etc., to save water in agriculture is critical and should be a priority research (Sadeghi-Shoae *et al*., 2013). Compared to other environmental stresses, water deficit limits the growth and the productively of crops (Yamaguchi-Shinozaki *et al*., 2002) Many previous studies have studied the effect of drought stress on different crops and attempted to explore efficient measure for reducing its undesirable impacts on yield and yield components. Water deficits induce a series of morphological and physiological changes in the sugar beet plant such as reduction in leaf area and photosynthesis. Senescence of old leaves may be accelerated under stress conditions, hence reducing leaf longevity (Brown *et al*., 1987). Improving crop yield under drought stress is one of the most important goals of plant breeding (Cattivelli *et al*., 2008). Chemical treatment and agronomical crop management practices have been utilized to reduce the water deficit effects (Manivannan *et al*., 2007), but the application of zeolite_to affected plants attracted little attention. One possible approach to reducing the effect of water deficit on plant productivity is through the addition of zeolite to soil. Zeolite is a group of naturally occurring minerals with physical and physicochemical properties that can be used in such diverse areas as construction and agriculture that can absorb and hold potentially harmful or toxic substances. It also is capable of absorbing part of the excessive nutrients and also water, resulting in more balanced macro nutrient cation ratios in the root environment and also can keep water in root zone (Sawas *et al*., 2004). Zahedi *et al*. (2009) reported that the zeolite application may improve plant growth under drought stress. By using the zeolites, we can preserve the moisture of the soil for long-term and get available to the plant, so the usage of the Zeolite can modify the effects of drought stress in the agricultural systems. Drought is one of the major environmental stresses that limit the growth of plants and the production of crops (Kavoosi, 2007). Desiccation affects microbial population structure (Ilyas *et al.,* 2008). Bacteria grown in places where water is limited or where dry periods occurs frequently, have been shown to promote plant growth better than those growing in sites where water is abundant (Mayak *et al*., 2004). Hashemi *et al*. (2014) reported water supply and Application of bio-fertilizers increased the sugar content and sugar yield in sugar beet, in other hand highest sugar yield and white sugar yield was belonged at application of bio-fertilizer (Biozar type) in Oct-13 irrigation closed time and minimum sugar yield and white sugar yield were belonged at application of bio-fertilizer (Nitrokara type) in Oct-21 irrigation closed time. Soil organisms play a crucial role in the functioning of soil agricultural ecosystems. The functions performed by the soil biota have major direct and indirect effects on soil quality, crop growth and quality, its disease resistance, and thus on the sustainability of crop production systems (Roger-Estrade *et al*., 2010). Jafarnia *et al*. (2013) reported biological fertilizer application in sugar beet could increase qualitative characteristics of sugar beets root and reduced consumption of chemical nitrogen fertilizer in both locations. Abo-El-Goud (2000) reported that using biological fertilizer had a positive impact on the weight of the fresh and dry root and the weight of the fresh and dry stem, as well as leaf surface indicator in wheat. Nitrobacteria presented in the soil environment of inoculated sugar beet seeds showed a significant effect on the secretion of additive growth substances such as Gibberellins (Mrkovacki *et al*., 2001). Favilli *et al*. (1993) inoculated sugar beet seed with a fertilizer containing a biological agent of Azosperillium accelerated the germination, seedling growth and optimum plant growth and increased root and sugar yield and reduce nitrogen fertilizer requirement during the growth season. Arbuscular mycorrhiza (AM) fungi symbiosis protects host plants against the detrimental effects of drought stress through mechanisms of drought avoidance (Ruiz-Sanchez *et al*., 2010). The colonization of roots by AM fungi in various plant species induces proline accumulation when water is limiting (Yooyongwech *et al*., 2013). The enhanced accumulation of proline in these studies was linked to AM-induced drought resistance in which proline acts as osmoprotectant. Conversely, in several studies, while proline content increased in response to water deficit, a lower accumulation of proline has been observed in mycorrhizal plants relative to non-mycorrhizal counterparts (Doubkova *et al*., 2013), suggesting that AM symbiosis enhanced host plant resistance to drought. Sustainable agriculture focuses on developing new comprehensive farming practices including management of soil microorganisms that are safe and environmentally friendly fostering the development of multi-disciplinary studies (Rashidi and Abbassi, 2011). Therefore, the aim of the present study was to investigate the effect of zeolite and biological fertilizers application on root yield and quantitative traits in sugar beet under different irrigation pattern.

MATERIALS AND METHODS

Field and Treatments Information

This research was conducted in Islamic Azad University- Karaj Branch located in North of Iran (Longitude 59′ 51° E, latitude 48′ 35° N and altitude of 1313 m above sea level) via split-split plot experiment based on randomized complete block design with four replication during 2011- 2012. The main factor included irrigation regime at two levels (normal and stress), sub factor included zeolite application at two levels (with and without application) and biological fertilizers at four levels [1- Non application of mycorrhiza, 2- Application of mycorrhiza 3- Non -inoculation of bacteria 4- Inoculation of bacteria (*Pseudomonas, Azotobacter, Azospirillum*)] belonged to sub-sub factor. The planting density was approximately 10 plant.m^{-2}. Every plot in field included 6 rows with length of 5m and distance of 60 cm between rows and 25 cm distance between plants. Before planting, soil sampling was conducted from 30 cm depth (Table 1).

Table 1. Physical and chemical properties of the research field

Depth (cm)	0-30
EC ($ds.m^{-1}$)	2.5
Soil acidity (pH)	7.6
O.C (%)	1.99
Phosphorous (ppm)	15.3
Potassium (ppm)	250.9
Soil texture	Loamy

Crop Management

According to soil test results, adequate amount of chemical fertilizer, urea (150 $kg.ha^{-1}$), the first half of which during harrowing in spring and the remaining half before hoeing when the plants reached the six leaf stage, triple superphosphate (100 $kg.ha^{-1}$) and potassium phosphate (150 $kg.ha^{-1}$) were applied uniformly. The sugar beet was established with furrow irrigation system. Weeds were controlled by hand weeding when necessary.

Traits Measure

Irrigation System: A gypsum block electrical conductivity system (Soil moisture meter, model: 5910A) was used for evaluating of plots for watering which is in accordance with calibration electrical conductivity graph of the soil introduced by Paknejad *et al*. (2009). Irrigation of plots performed regularly when 75% and 40% of moisture evacuated from soil in stress and normal plots, respectively. Harvesting was started on 16th of November. Sugar content was measured by polarymeteric method by Sacchary meter device and sodium, potassium and nitrogen was measured by betalizer device (Payne, 1968; Rinaldi and Alessandro. 2006; Wittenmayer and Schilling. 1998).

Quantitative and qualitative traits: To measure root yield, all plants were harvested from 4.8 m^2 area and shoot and root were divided. Roots were counted and transferred to the Sugar Technology Laboratory. In the laboratory, roots were weighted before providing pulp from them. White sugar yield was obtained by multiplying root yield × sugar percentage. White sugar yield is the most important parameter in sugar beet production which is the amount of sugar that can be extracted from roots. It is always lower than the total sugar yield (Cooke and Scott, 1993). To obtain sugar content, the Polarimetry method by Saccharomat instrument was used which is the most common method (Clover *et al*., 1998). Sodium and potassium contents were measured by flame photometry method. Moreover, α-amino N was measured by betalizer device. (Clover *et al.,* 1998).

Statistical Analysis

Data were analyzed by SAS software (Ver. 8). Mean comparisons were conducted by Duncan multiple range test at 5% probability level.

RESULTS AND DISCUSSION

Root yield

Result of analysis of variance showed effect of irrigation regime and bio-fertilizer on root yield was significant at 5% probability level but effect of zeolite was not significant although interaction effect of all treatments was significant at 1% probability level (Table 2). Mean comparison interaction effect of treatments showed the highest and the lowest amount of root yield belonged to the $NZ_1M_1B_0$ (Zeolite and mycorrhiza application and non-inoculation of bacteria under normal irrigation regime) (73340 $kg.ha^{-1}$) and $DZ_1M_0B_0$ (Zeolite application and non-application of mycorrhiza and bacteria under stress irrigation regime) (38200 kg.ha^{-1}) (Fig.1). Therefore zeolite and mycorrhiza application together has a positive impact on root yield. Although a possible approach to reducing the effect of water deficit on plant productivity is through the addition of zeolite to soil but in this treatment zeolite application alone had no effect on root yield. Another researcher confirmed that result (Ruiz-Lozano and Aroca, 2010). Kenter *et al.* (2006) concluded that irrigation (soil water content) had no significant influence on leaf growth rate but root growth rate increased significantly with increasing soil moisture content. Wittenmayer and Schilling (1998) mentioned that if sugar beet is subjected to water stress, the root yield would decrease.

Table 2. Analysis of variance result of measured traits

S.O.V	df	Root yield	White sugar content	Sugar content	Potassium content	Nitrogen content	Sodium content
Replication	3	1.002	1.437	1.092	1.816	1.601	15.013
Irrigation regime (I)	1	27.641*	39.911*	25.531*	13.969**	8.115**	55.465*
Error I	3	2.945	5.599	3.345	0.551	0.947	3.401
Zeolite (Z)	1	0.019^{ns}	0.706*	0.013*	0.429^{ns}	0.914**	6.175*
Error II	1	51.266	72.463	41.266	5.99	1.473	65.934
Biofertilizer (BF)	3	1.908*	2.749*	1.467^{ns}	0.181**	0.247**	0.986*
I * Z	1	0.487^{ns}	14.437^{ns}	10.487^{ns}	6.634^{ns}	0.0186^{ns}	0.0407^{ns}
I * BF	3	3.732^{ns}	10.008^{ns}	1.512^{ns}	0.526**	0.944*	0.0409^{ns}
Z * BF	3	23.621^{ns}	0.0529^{ns}	21.721^{ns}	0.0212^{ns}	0.107*	3.178^{ns}
I * BF* Z	3	0.412**	19.729*	0.0314**	0.03.31^{ns}	0.0116^{ns}	0.0132*
Error III	15	3.959	10.04	3.356	0.306	0.793	21.043
CV (%)	-	9.7	7.1	8.1	11.17	10.2	9.2

$^{ns,\ *}$ and **: no significant, significant at 5% and 1% of probability level, respectively.

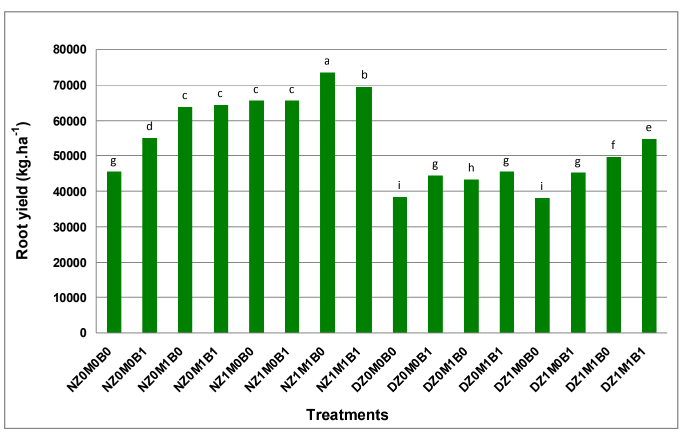

Fig. 1. Mean comparison interaction effect of irrigation regime, Zeolite and bio-fertilizer treatments on root yield via Duncan test at 5% probability level. N: Normal stress, D: Drought stress, Z_0: Non application of zeolite, Z_1: Zeolite application, M_0: Non application of mycorrhiza, M_1: Mycorrhiza application, B_0: Non-inoculation of bacteria, B_1: Inoculation of bacteria (Pseudomonas, Azotobacter and Azospirillum).

The symbiotic relationship between arbuscular mycorrhizal (AM) fungi and the roots of higher plants is widespread in nature, and several Eco physiological studies have demonstrated that AM symbiosis is a key component for assisting plants to cope with water stress and increasing drought resistance, as demonstrated in a number of host plant and fungal species (Ruiz-Lozano, 2003).

White sugar content (WSC)

According result of analysis of variance effect of irrigation regime, zeolite, bio-fertilizer and interaction effect of all treatments on white sugar content was significant at 5% probability level (Table 2). Mean comparison interaction effect of treatments indicated the highest and the lowest amount of WSC belonged to the $NZ_1M_1B_0$ (Zeolite and mycorrhiza application and the non-inoculation of bacteria under the normal irrigation regime) (11.88%) and the $DZ_1M_0B_0$ (Zeolite application and non-application of mycorrhiza and bacterial under stress irrigation regime) (5.46%) (Fig. 2). Rinaldi and Alessandro (2006) and Tohidi moghadam *et al*. (2009) reported same result. Mahmoodi *et al*. (2008) showed that the optimum soil water content for root yield is 70% of field capacity by 78.5 $t.ha^{-1}$. The minimum root yield (52.5 $t.ha^{-1}$) was observed at 90% of field capacity.

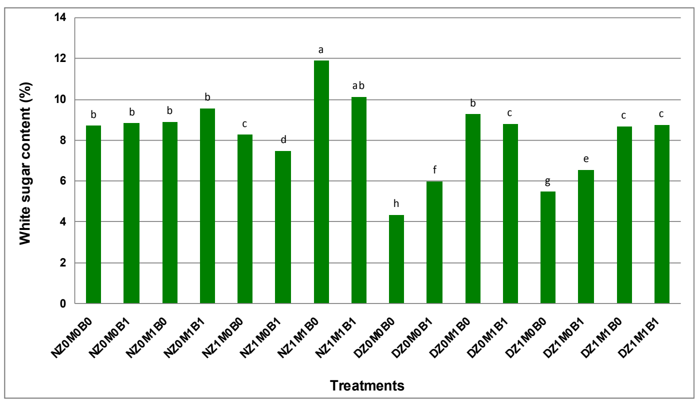

Fig. 2. Mean comparison interaction effect of irrigation regime, Zeolite and bio-fertilizer treatments on white sugar content via Duncan test at 5% probability level. N: Normal stress, D: Drought stress, Z_0: Non application of zeolite, Z_1: Zeolite application, M_0: Non application of mycorrhiza, M_1: Mycorrhiza application, B_0: Non-inoculation of bacteria, B_1: Inoculation of bacteria (Pseudomonas, Azotobacter and Azospirillum).

Irrigation at 30%, 50 and 70% of field capacity had some impacts on sugar content while sugar content decreased at 90% field capacity when the available soil water content was at 70% of field capacity, maximum root yield and quality was observed (Ober, 2001). Zahedi and Tohidi-Moghadam (2011) reported that zeolite application in soil decreased antioxidant enzymes activity. It seems that zeolite increases water retention capacity and thus water stress intensity will be decreased.

Sugar content (SC)

Result of analysis of variance revealed effect of irrigation regime and zeolite on sugar content was significant at 5% probability level but effect of bio-fertilizer was not significant, although interaction effect of all treatments was significant at 1% probability level (Table 2). Mean comparison interaction effect of treatments showed the highest and the lowest amount of SC belonged to $NZ_1M_1B_0$ (Zeolite and mycorrhiza application and non-inoculation of bacteria under normal irrigation regime) (16.47%) and the $DZ_0M_0B_0$ (Non-application of zeolite, mycorrhiza and bacteria under stress irrigation regime) (8.7%) (Fig.3). The results were in similar with the finding of other researchers (Mahmoodi *et al*., 2008; Soltanmorad *et al*., 2015; Rashidi and Abbassi. 2011).

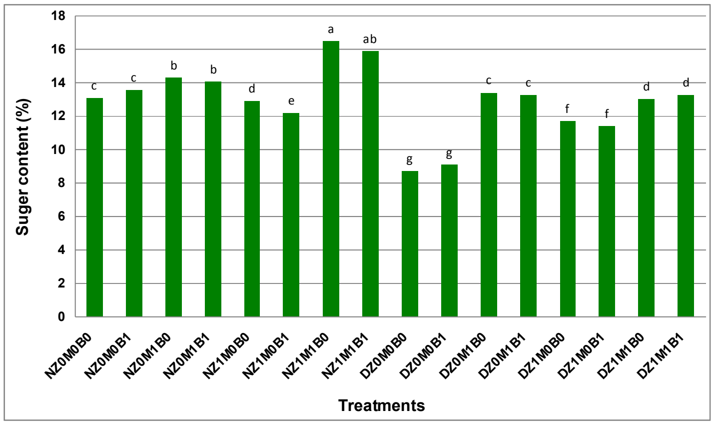

Fig. 3. Mean comparison interaction effect of irrigation regime, Zeolite and bio-fertilizer treatments on sugar content via Duncan test at 5% probability level. N: Normal stress, D: Drought stress, Z_0: Non application of zeolite, Z_1: Zeolite application, M_0: Non application of mycorrhiza, M_1: Mycorrhiza application, B_0: Non-inoculation of bacteria, B_1: Inoculation of bacteria (Pseudomonas, Azotobacter and Azospirillum).

Potassium content

According the result of analysis of variance effect of irrigation regime, bio-fertilizer and interaction effect of treatments (Irrigation regime × bio fertilizer) on the potassium content was significant at 1% probability level, but effect of another factor was not significant (Table 2).

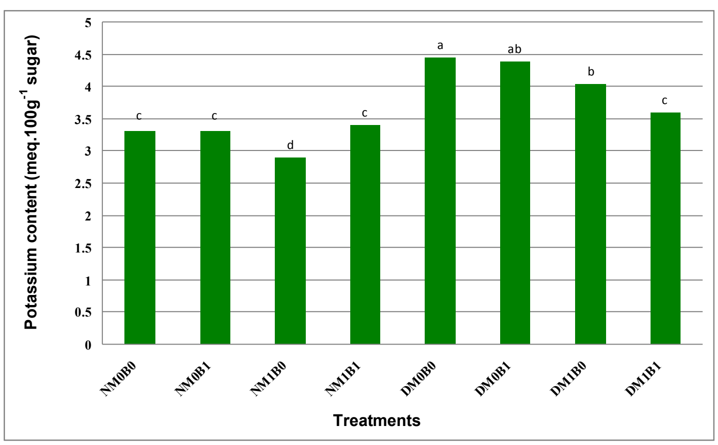

Fig. 4. Mean comparison interaction effect of irrigation regime, Zeolite and bio-fertilizer treatments on potassium content via Duncan test at 5% probability level. N: Normal stress, D: Drought stress, Z_0: Non application of zeolite, Z_1: Zeolite application, M_0: Non application of mycorrhiza, M_1: Mycorrhiza application, B_0: Non-inoculation of bacteria, B_1: Inoculation of bacteria (Pseudomonas, Azotobacter and Azospirillum).

Mean comparison results of interaction effect of irrigation regime × biofertilizer indicated that highest and the lowest amount of potassium content belonged to DM_0B_0 (Non application of mycorrhiza and bacteria under stress irrigation regime) (4.45 meq.100g^{-1} sugar) and NM_1B_0 (Mycorrhiza application and non inoculation of bacteria under normal irrigation) (2.89 meq.100g^{-1} sugar) (Fig. 4). Another researchers such as El-Ghareib *et al*. (2012) reported same result.

Amino-nitrogen content

Result of analysis of variance revealed effect of irrigation regime, zeolite and bio-fertilizer on amino-nitrogen content was significant at 1% probability level although interaction effect of irrigation regime × biofertilizer and zeolite × bio-fertilizer on mention trait was significant at 5% probability level, but effect of another factor was not significant (Table 2).

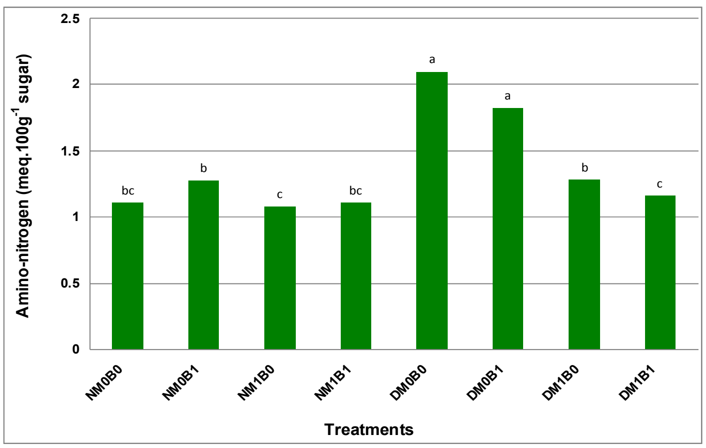

Fig. 5. Mean comparison interaction effect of irrigation regime, Zeolite and biofertilizer treatments on amino-nitrogen via Duncan test at 5% probability level. N: Normal stress, D: Drought stress, M_0: Non application of mycorrhiza, M_1: Mycorrhiza application, B_0: Non-inoculation of bacteria, B_1: Inoculation of bacteria (Pseudomonas, Azotobacter and Azospirillum).

The study of mean comparison of irrigation regime and biofertilizer showed that the highest and the lowest amount of amino-nitrogen content belonged to DM_0B_0 (Non-application of mycorrhiza and bacteria under stress irrigation regime) (2.09 meq.100g^{-1} sugar) and NM_1B_0 (Mycorrhiza application and non-inoculation of bacteria under normal irrigation regime) (1.07 meq.100g^{-1} sugar) (Fig. 5). According result of mean comparison of zeolite and biofertilizer the highest and the lowest amount of amino-nitrogen content belonged to $Z_0M_0B_0$ (Non-application of zeolite, mycorrhiza and bacteria) (1.93 meq.100g^{-1} sugar) and $Z_1M_1B_0$ (Zeolite and mycorrhiza application and non-inoculation of bacteria) (1.03 meq.100g^{-1} sugar) (Fig. 6). Another researcher confirmed that result (Jafarnia *et al*., 2013; Rassam *et al*., 2015; El-Fouly *et al*., 2005). Previous studies represented that drought consistently influenced the quality of sugar beet by increasing impurities such as alfo-amino, nitrogen, sodium, potassium and decreasing extractable sugar (Amin *et al*., 2013). Clover *et al*. (1999) claimed that drought had a remarkable effect on the concentration of alfa-amino nitrogen in the storage root but had little effect on potassium and sodium contents.

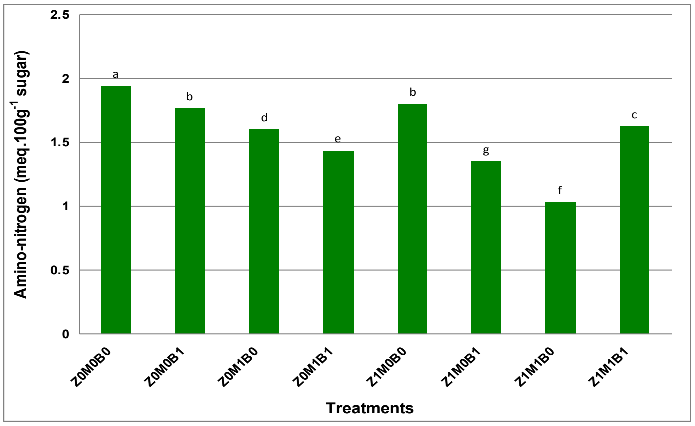

Fig. 6. Mean comparison interaction effect of irrigation regime, Zeolite and bio-fertilizer treatments on amino-nitrogen content via Duncan test at 5% probability level. Z_0: Non application of zeolite, Z_1: Zeolite application, M_0: Non application of mycorrhiza, M_1: Mycorrhiza application, B_0: Non-inoculation of bacteria, B_1: Inoculation of bacteria (Pseudomonas, Azotobacter and Azospirillum).

Sodium content

According result of analysis of variance effect of irrigation regime, zeolite, bio-fertilizer and interaction effect of all treatments on sodium content was significant at 5% probability level (Table 2). Mean comparison interaction effect of treatments indicated that the highest and the lowest amount of sodium content belonged to $DZ_0M_0B_0$ (Non-application of zeolite, mycorrhiza and bacterial under stress irrigation regime) (11.95 meq.100g^{-1} sugar) and $NZ_1M_1B_0$ (Zeolite and mycorrhiza application and non-inoculation of bacteria under normal irrigation regime) (5.09 meq.100g^{-1} sugar) (Fig. 7). The results were in similar with the finding of other researchers (Ghareib and El-Henawy, 2011; Farnia and Hashemi, 2015).

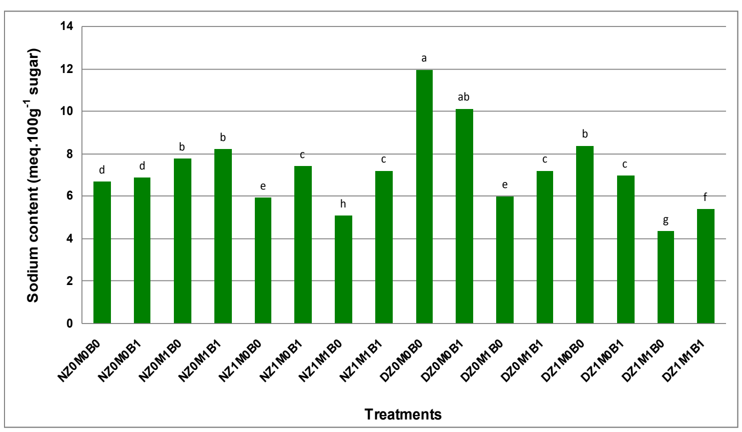

Fig. 7. Mean comparison interaction effect of irrigation regime, Zeolite and bio-fertilizer treatments on sodium content via Duncan test at 5% probability level. N: Normal stress, D: Drought stress, Z_0: Non application of zeolite, Z_1: Zeolite application, M_0: Non application of mycorrhiza, M_1: Mycorrhiza application, B_0: Non-inoculation of bacteria, B_1: Inoculation of bacteria (Pseudomonas, Azotobacter and Azospirillum).

CONCLUSIONS

Use of growth promoting bacteria showed efficient role in decreasing harmful nutrients in sugar beet. Zeolite and mycorrhiza application together, under normal irrigation regime increased root yield, sugar yield and white sugar content, also had lowest potassium, sodium and amino-nitrogen. In the drought condition quantitative triat decreased comparing with well irrigated treatment and this result could be attributed to soil moisture, which affects the movement of nutrient in the soil. Zeolite and mycorrhiza application can improve growth conditions for sugar beet plants grown under water deficit stress. 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. Therefore such materials can reduce losing soil moisture in arid and semi-arid regions by soil physical improvement.

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