

BIOZEOLITE FOR IMPROVING BEAN PRODUCTION UNDER ABIOTIC STRESS CONDITIONS

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Abstract: *Alleviating the adverse effect of drought and salinity is an important approach especially if climate change scenarios are realized. A Field experiment was carried out at Ismailia Governorate, Egypt to study the effect of zeolite (clinoptilolite as a most common type of zeolites minerals) and biozeolite (biofertilizer-zeolite mixture at rate of BZ0, BZ1, BZ2, BZ3 and BZ4) for improving faba bean production under salinity (EC of soil = 8.2 dSm⁻¹) and water stress (I1= 100% and I2= 85% of water requirements) compared to sole application of the biofertilizer and different rates of zeolite (Z0, Z1, Z2, Z3 and Z4). Under studied field conditions which are characterized by salinity and sandy texture, control plots recorded low values in most studied trails as a reflection to salinity stress. While applying both zeolite and biozeolite alleviated salinity stress where high significant increases in growth parameters and yield of bean exist. Irrespective of control (Z0) and single addition of biofertilizer (BZ0), application of zeolite increases the growth parameters following the order: Z4>Z3>Z2>Z1 under both irrigation rates; I1>I2. On the other hand, all nutrient concentrations record an increase in case of low water amount except nitrogen. The maximum values of nutrient use efficiency (NPK-UE), irrigation water use efficiency (IWUE) and economic water productivity (EWP) were associated with the interactions (I1 x BZ4), (I2 x BZ3) and (I2 x BZ4). The chemical composition (total carbohydrates, total phenolic, flavonoid and tannins) of seeds were determined. In addition to N, P, K, Ca, Mg and Na content (kg/fed.), K/Na, Ca/Na and Mg/Na ratios were calculated.*

Key words: *Biofertilization- zeolite- salt affected soils- nutrients-chemical composition*

1. Introduction

Salinity and drought are the most

poignant environmental stresses which cause huge losses to agricultural production and consequently affect the

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economic strengths of countries. Soil salinity is the most widespread abiotic stress in the arid and semiarid regions. Its severity is expected to increase if climate change scenarios are realized. Salinity is responsible in decreasing crop production, water availability for plants, ion-specific toxicities or imbalance, decreasing nutrients uptake, osmotic imbalance and thus, affecting plant physiology [3]. Soil salinity control is to avoid the degradation resulted by salt accumulation in soil and recovering it by remediation processes [42]. Living with salt stress by utilizing distinctive soil management and how to deal with salinity is considered a significant practice in improving agricultural production within the reclamation period [1].

Egypt is characterized by arid condition; high evapotranspiration, high temperature and low rainfall, also suffering from insufficient water supply for irrigation especially in new cultivated areas [19], [28]. Inadequate water supply resulted in biochemical changes in plants like accumulation of some organic compounds [14] and promotes strong decrease in stomatal conductance [34]. As a result of the expanding demand for freshwater supplies with the exhaustion of water sources, farmers are compelled to utilize either effective irrigation water systems or decreased water application rates. Accordingly, scientists, agriculturists, and decision makers should consider the conceivable methods for lessening the additive water quantity and raising irrigation water use efficiency [46], [30].

Zeolites are hydrated aluminosilicates, have the ability to exchange some of their constituent cations, that depending upon the amount of Al^{3+} that replaces Si^{4+} in the structure, so zeolites group has high cation exchange capacity, and thus it can influence the pedo-chemical environment during soils formation [38]. Zeolite

(clinoptilolite) improves nutrient retention without degradation for an extended time. Its addition to the soil may retain beneficial nutrients and reserve water in rhizosphere, consequently, reduce water and fertilizer application rates and costs. Furthermore, zeolite's porous structure keeps the soil moist, aerated, and active over time [41]. Soil application of zeolite is ameliorating salinity stress and improving nutrient balance by 1) assisting water infiltration and retention by the capillary pores, 2) increasing water holding capacity of the soil and accumulated more salts [5], [6].

Biofertilizer is a substance which contains living microorganisms; of no toxic effect on soil. The use of biofertilizer is of low cost when compared to chemical fertilizer [8]. Moreover, the biofertilizer promotes plant growth and productivity and has internationally been accepted as an alternative source of chemical fertilizer. Rhizobacteria increases plant growth effectively because of colonizing plant root led to producing growth hormones, raising nitrogen fixation rate, phosphorus-solubilizing activity and biological control activity [17]. Bacteria of family Rhizobiaceae are symbiotic and effectively convert atmospheric nitrogen which is utilized by the host [37], [16].

Thus, the integration between biofertilization and zeolite in alleviating faba bean to abiotic stress (salinity and water stress) compared to sole application of the biofertilizer and different rates of zeolite is the main purpose of this study.

2. Materials and Methods

2.1. Materials

A field experiment was carried out at a private farm (30° 21' 54.30" N and 31° 52' 43.93" E) in Ismailia Governorate, Egypt during winter season (2014) to study the effect of zeolite (clinoptilolite) which was

applied in different rates in presence (with constant mixing ratio) or absence of biofertilizer (Okadien as a most popular biofertilizer in Egypt) on faba bean production and its ability to alleviating abiotic stress (salinity and water stress). Bean cultivar (*Vicia faba* L. cv. Giza 716) was obtained from Ministry of Agriculture, Giza, Egypt. The experiment included the following treatments: A) two irrigation rates (I1 = 100% of full water requirement and I2= 85% of full water requirement, B) five rates of zeolite (Z0=control, Z1=75, Z2=150, Z3=300, Z4=450 kg/fed.) were applied twice with and without the biofertilizer.

All amendments were manually located in furrows beside irrigation lines.

The fertilization requirements and all agricultural practices were followed according to the recommendations of

Ministry of Agriculture in this district and no pesticides were used.

Water requirement was calculated using average meteorological parameters (2003-2013) using CROPWAT computer model [21] (according to the climatic data recorded at Ismailia Weather Station), based on calculation of Penman Monteith equation and the Kc values presented in the program and also illustrated in [7]. Crop evapotranspiration (ETc) was calculated according to the following formula: $ET_c = K_c \times ET_0$ (ET_c = crop evapotranspiration in mm/day, ET_0 = reference evapotranspiration in mm/day and K_c = crop coefficient). Drip irrigation system was used with 50 cm-spaced emitters with a flow rate of 4 Lh⁻¹, three days irrigation interval and the water requirements of bean plants is presented in Table 1.

Table 1

Water requirements for drip irrigated bean grown on a sandy soil at Ismailia governorate

Month	Nov.	Dec.	Jan.	Feb.	March	Total		
Period	25-30	1-31	1-31	1-28	1-25			
ET ₀ mm/day	3.2	2.1	1.5	2.2	2.7			
No. of days	5	31	4	30	10	18	22	120
Kc	0.5		1.15		0.3			
ETc mm	8	32.55	3	51.75	25.3	11.88	17.82	
Eu	90%							
Lr	10%							
R m ³ /season/fed. (I ₁)	41.5	168.8	15.6	268.3	131.2	61.6	92.4	
	225.8		399.5		154.0		779.3	

ET₀ (reference evapotranspiration), Kc (crop coefficient), Eu (application uniformity), Lr (leaching requirements), IR (irrigation requirements), I₁ (100% of water requirements).

Irrigation water use efficiency was calculated for each treatment using the following formula

$$IWUE = \frac{\text{Yield (seeds yield as kg/fed.)}}{\text{Total water applied (m}^3\text{/fed.)}} = \text{kg/m}^3$$

Initial soil sample were air dried, crushed, sieved to pass through 2mm sieve and analyzed for their physical and chemical characteristics as described by [32], [39]. Some physical and chemical properties of the studied soil are given in Table 2.

Physical and chemical properties of the studied soil

Table 2

pH 1:2.5	ECe dSm ⁻¹	Cations and anions meq./L								Particles size distribution (%)			Texture
		Na ⁺	K ⁺	Ca ⁺⁺	Mg ⁺⁺	CO ₃ ⁻⁻	HCO ₃ ⁻	Cl ⁻	SO ₄ ⁻⁻	Sand	Silt	Clay	
7.83	8.2	57.5	0.3	17.3	6.9	-	1.6	78.2	2.2	74.1	16.2	9.7	Sandy

Grayish-white zeolite (Clinoptilolite) has from Alex Company, Egypt 99.3% of grain size < 2mm was obtained (<http://alixzeolite.com>) (Table 3).

Physical and chemical properties of the studied natural zeolite

Table 3

pH 1:2.5	EC dSm ⁻¹ 1:5	Cations and anions g./L							
		Na ⁺	K ⁺	Ca ⁺⁺	Mg ⁺⁺	CO ₃ ⁻⁻	HCO ₃ ⁻	Cl ⁻	SO ₄ ⁻⁻
7.5	0.43	2.2	0.3	1.7	0.8	-	0.3	2.8	1.9
WHC %	BD gcm ⁻³	CEC meq/100g	SP %	Particle size distribution					
				2-1 mm	1-0.5 mm	0.2-0.25 mm	0.25- 0.125mm	0.125- 0.063mm	<0.063 mm
62	1.1	145	45	24.28	38.46	0.20	13.44	9.25	14.37

Scanning electron microscope SEM and transmission electron microscope TEM of zeolite were measured using Quanta FEG 250 a scanning electron microscope and JEOL JEM-2100 a high-resolution transmission electron microscopy for TEM, respectively.

At harvesting, bean plants were carefully removed at approximately 2.5 cm above the soil from one meter and dried to constant weight. Plant response was determined by measuring the following parameters: number of pods/plant, straw, pods, seeds and biological yields (kg/fed.). Portions of these dried seeds were ground and wet-digested with di-acid mixtures, the digested aliquot was analyzed for macronutrients (N, P, K, Ca, Na and Mg) as described by [15]. The concentrations (%) and content (kg/fed.) of all mentioned macronutrients, its relations with Na, NPK use efficiency (NUE, PUE and KUE), harvest index (HI=seed yield/biological yield*100), economic water productivity (EWP= income from the sale of seeds (EL)/irrigation water quantity (m³) [9], [2] were calculated. The chemical composition of bean seeds was determined as follows:

2.2. Preparation of Seed Extract

The dried bean seed were ground, powdered, stored at 4 °C and protected from light prior to further use. Ten g of the dried powder from skin and peel were soaked with 80% ethanol, and shaking at room temperature for 48 h. The extracts were filtered and the extraction was repeated twice. The different extracts were used for the determination of total phenolic, flavonoid, and tannins using spectrophotometer (Uicum UV 300). The total phenolic content (TPC) of bean seeds extracts were spectrophotometrically determined by Folin-Ciocalteu reagent assay using gallic acid for the preparation of calibration curve (20 – 120 mg/l) according to the method of [43]. A suitable aliquot (1 ml) of each extract or standard solution was added to 25 ml volumetric flask, containing 9 ml of distilled water. Total flavonoid content (TFC) of bean seeds was spectrophotometrically determined by the aluminum chloride method using quercetin as a standard [45]. Total tannins content (TTC) of bean seeds

was measured using the Folin-Ciocalteu reagent assay according to [40].

The total hydrolysable carbohydrates were spectrophotometrically determined using phenol- sulphuric acid method [18].

The experimental treatments were replicated three times in split plot design with irrigation treatments in main plots and different amendments in subplots. All data obtained from this study were statistically analyzed through analysis of variance (ANOVA) and the least significant difference (LSD) at 0.05 probability level was applied to make comparisons among treatment means according to [23].

3. Results

3.1. SEM and TEM of used zeolite

Scanning electron microscope (SEM) and transmission electron microscope (TEM) were used to observe the morphology and some characteristics of fine powder of zeolite (clinoptilolite).

Fig. 1 illustrated that zeolite has several pores and uniform deep channels between pure crystals that arranged in a lamellar composition with geometric shape. These channels are formed by tetrahedral rings. Most particles similar in size as well as pores have the same diameter, approximately. These properties may confirm the high cations exchange capacity, high water holding capacity and low bulk density of zeolite as shown in Table 3. These findings were in close agreement with those obtained by [33] as they reported that the pores between crystal aggregates of clinoptilolite are similar and up to 500 nm.

The integrated effects of applying different zeolite and biozeolite rates on faba bean growth, yield, nutrient concentrations and content of seeds, nutrient use efficiency, seeds chemical

composition, irrigation water use efficiency and economic water productivity under two different irrigation rates will be discuss as follows.

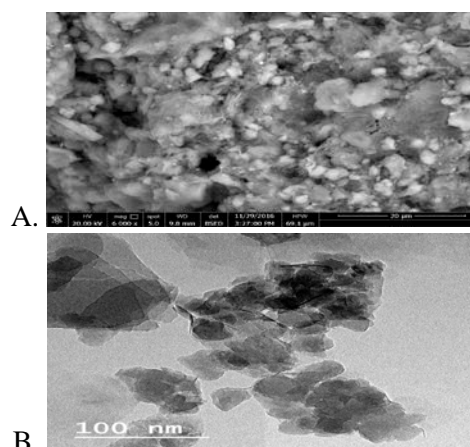


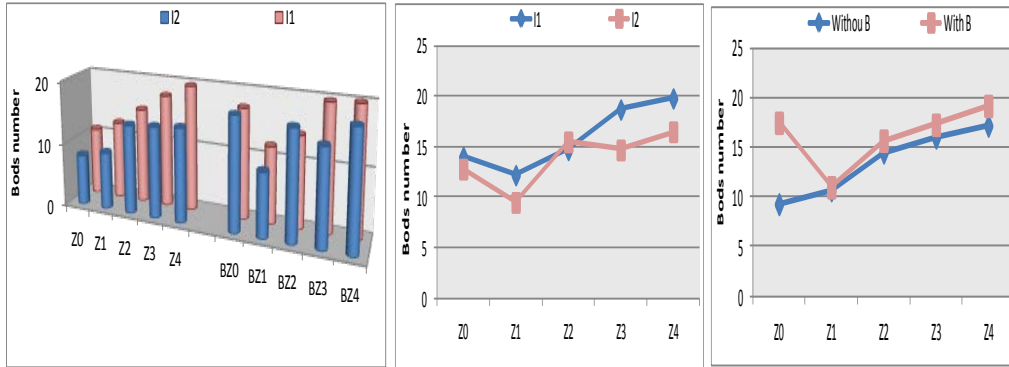
Fig. 1. SEM (A) and TEM (B) of used zeolite

3.2. Growth Parameters

The three-way analysis of irrigation rates, zeolite addition and biofertilizer on bean growth parameters; pods number/plant, straw and pods yield are illustrated in Fig. 2, 3 and 4, whereas seeds and biological yields (kg/fed.) are given in Tables 4 and 5.

All studied growth parameters increased significantly by the addition of all water requirements compared to water stress treatment.

Irrespective of control treatments (Z0 and BZ0), application of zeolite increases the growth parameters following the order of: Z4>Z3>Z2>Z1. Regardless of zeolite rates, application of BZ enhances all values significantly compared to addition of zeolite only by 19.3, 19.1, 22.6, 19.6 and 20.9% for pods number, straw, pods, seeds and biological yields, respectively.



Note: For this figure and subsequent figures and tables: $I_1=100\%$ and $I_2= 85\%$ of water requirements, Z0, Z1, Z2, Z3 and Z4 denote zeolite application rates of 0, 75, 150, 300 and 450 kg/fed., respectively. "BZ" denotes treated soil with biofertilizer-zeolite mixture.

Fig. 2. The integrated effect of zeolite and biofertilizer on pods number under different irrigation rates

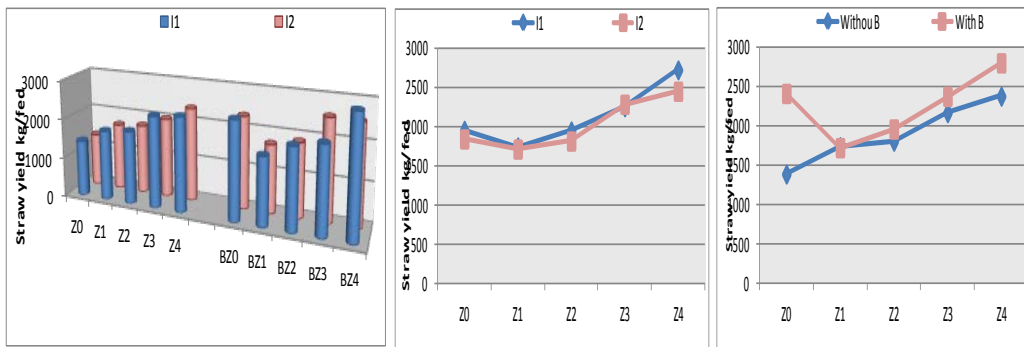


Fig. 3. The integrated effect of zeolite and biofertilizer on straw yield under different irrigation rates

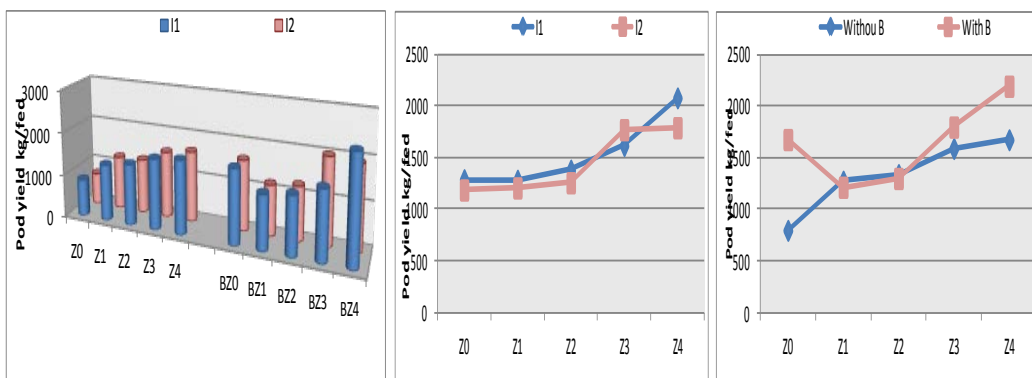


Fig. 4. The integrated effect of zeolite and biofertilizer on pods yield (kg/fed.) under different irrigation rates

Table 4
The integrated effect of zeolite and biofertilizer on seed yield (kg/fed) under different irrigation rates

Amendments	Irr. Rate		Mean	
	I ₁	I ₂		
Z0	714	652	683	
Z1	1067	958	1013	
Z2	1153	1022	1088	
Z3	1465	1281	1373	
Z4	1543	1308	1426	
Mean	1188	1044	1116	
BZ0	1341	1245	1293	
BZ1	1029	988	1009	
BZ2	1010	1031	1021	
BZ3	1326	1583	1455	
BZ4	2106	1686	1896	
Mean	1362	1307	1335	
Z mean	Z0	1028	949	988
	Z1	1048	973	1011
	Z2	1082	1027	1054
	Z3	1396	1432	1414
	Z4	1825	1497	1661
Mean	1275	1175		
LSD _{0.05}	I=**70.8 B=***81.8 Z=***168.6 ZxB=*** IxZ=* IxB=ns IxBxZ=ns			

The individual application of biofertilizer increases the studied parameters with high percentage. Although, the mixture of low rates (BZ1 and BZ2) increases all growth values compared to Z0, it decreases the same parameters compared to BZ0 and tend to increase by increasing addition rates (BZ3 and BZ4). Both of Z4 and BZ0 increase pods number, straw, pods and biological yields by 1.8, 1.7, 2.1 and 1.9 time compared to control (Z0), respectively, while the mixture at high rate BZ4 augmented these parameters by 2.1, 2.0, 2.8 and 2.3 times in the same pattern, irrespective of irrigation rates. As for seed yield, the high rate of zeolite Z4 was better than application of biofertilizer alone (BZ0) where they raised yield by 2.1 and 1.9 time, respectively, while the superiority

Table 5
The integrated effect of zeolite and biofertilizer on biological yield (kg/fed.) under different irrigation rates

Amendments	Irr. Rate		Mean	
	I ₁	I ₂		
Z0	2280	2081	2181	
Z1	3074	2937	3006	
Z2	3074	3020	3047	
Z3	3954	3554	3754	
Z4	4090	4007	4049	
Mean	3294	3120	3207	
BZ0	4198	3974	4086	
BZ1	2877	2899	2888	
BZ2	3391	3122	3257	
BZ3	3781	4555	4168	
BZ4	5511	4473	4992	
Mean	3952	3805	3878	
Z mean	Z0	3239	3028	3133
	Z1	2976	2918	2947
	Z2	3233	3071	3152
	Z3	3868	4055	3961
	Z4	4801	4240	4520
Mean	3623	3462		
LSD _{0.05}	I=*145 B=***247 Z=***398 ZxB=** IxZ=* IxB=ns IxBxZ=**457			

was for BZ4 which amplified seed yield by 2.8 time compared to control.

The third interaction was not significant as for pods number and seed yield but was significant for straw, pods and biological yields. The highest values were obtained by application of biozeolite with high rate under irrigation with all water requirement and the lowest were recorded in control which was irrigated by low water quantity.

3.3. Nutrient Concentrations

The concentrations of N, Ca, Na, Mg were not affected significantly with all amendments and the interaction between them, while, P concentration was significantly affected with water quantity. Both the irrigation rates and

biofertilization affected K concentration also with significant difference (Tables 6 and 7).

In contrast to growth parameters, all nutrient values % record an increase with low water amount except nitrogen which confirms the results produced by yield as previously shown. Okadien application with or without zeolite led to increase K concentration compared to all zeolite

addition rates. Irrespective of the general mean value of control (Z0), N and Na concentrations were decreased, while, the reverse was true for K% and both of P and Ca concentrations were inconsistent.

Highest concentration of Na related to control treatment under lowest water quantity (Z0 x I2) and shortest amount of mentioned element was seen in the interactions (BZ3 x I1) and (BZ4 x I2).

Table 6
The integrated effect of zeolite and biofertilizer on N, P and K concentration (%) in bean seeds under different irrigation rates

Amendments	N			P			K			
	Irr. Rate		Mean	Irr. Rate		Mean	Irr. Rate		Mean	
	I ₁	I ₂		I ₁	I ₂		I ₁	I ₂		
Z0	3.75	4.09	3.92	0.09	0.26	0.18	1.31	1.54	1.43	
Z1	3.75	4.08	3.92	0.17	0.26	0.22	1.38	1.54	1.46	
Z2	3.64	3.75	3.70	0.11	0.22	0.17	1.33	1.48	1.41	
Z3	3.68	3.65	3.67	0.23	0.21	0.22	1.53	1.52	1.53	
Z4	3.47	3.31	3.39	0.23	0.25	0.24	1.55	1.46	1.51	
Mean	3.66	3.78	3.72	0.17	0.24	0.20	1.42	1.51	1.46	
BZ0	3.99	3.88	3.94	0.16	0.25	0.21	1.56	1.56	1.56	
BZ1	3.78	3.63	3.71	0.21	0.16	0.19	1.44	1.53	1.49	
BZ2	4.22	3.76	3.99	0.31	0.21	0.26	1.64	1.57	1.61	
BZ3	3.68	3.77	3.73	0.13	0.17	0.15	1.55	1.63	1.59	
BZ4	3.87	3.48	3.68	0.17	0.21	0.19	1.51	1.48	1.50	
Mean	3.91	3.70	3.81	0.20	0.20	0.20	1.54	1.55	1.55	
Z mean	Z0	3.87	3.99	3.93	0.13	0.26	0.19	1.44	1.55	1.49
	Z1	3.77	3.86	3.81	0.19	0.21	0.20	1.41	1.54	1.47
	Z2	3.93	3.76	3.84	0.21	0.22	0.21	1.49	1.53	1.51
	Z3	3.68	3.71	3.70	0.18	0.19	0.19	1.54	1.58	1.56
	Z4	3.67	3.40	3.53	0.20	0.23	0.22	1.53	1.47	1.50
Mean	3.78	3.74		0.18	0.22		1.48	1.53		
LSD _{0.05}	I=ns B=ns Z=ns ZxB=ns IxZ=ns IxB=* IxBxZ=ns			I=**0.03 B=ns Z=ns ZxB=** IxZ=* IxB=* IxBxZ=*0.9			I=*0.1 B=**0.04 Z=ns ZxO=* IxZ=ns IxO=ns IxOxZ=ns			

3.4. Nutrient Ratios to Na

All studied nutrient ratios with Na (K/Na, Ca/Na and Mg/Na) were enhanced

by using I2 rate compared to applied water requirement I1, especially Ca/Na where the differences between both irrigation rates were significant (Table 8).

Table 7

The integrated effect of zeolite and biofertilizer on Ca, Mg and Na concentration (%) in bean seeds under different irrigation rates

Amendments	Ca			Mg			Na			
	Irr. Rate		Mean	Irr. Rate		Mean	Irr. Rate		Mean	
	I ₁	I ₂		I ₁	I ₂		I ₁	I ₂		
Z0	0.28	0.32	0.30	0.16	0.14	0.15	0.13	0.20	0.17	
Z1	0.32	0.28	0.30	0.16	0.17	0.17	0.17	0.14	0.16	
Z2	0.25	0.30	0.28	0.16	0.19	0.18	0.15	0.16	0.16	
Z3	0.29	0.37	0.33	0.14	0.16	0.15	0.15	0.17	0.16	
Z4	0.25	0.30	0.28	0.12	0.16	0.14	0.15	0.13	0.14	
Mean	0.28	0.31	0.30	0.15	0.16	0.16	0.15	0.16	0.16	
BZ0	0.34	0.31	0.33	0.16	0.17	0.17	0.18	0.13	0.16	
BZ1	0.30	0.30	0.30	0.15	0.16	0.16	0.17	0.16	0.17	
BZ2	0.31	0.31	0.31	0.17	0.16	0.17	0.16	0.14	0.15	
BZ3	0.28	0.30	0.29	0.16	0.19	0.18	0.12	0.14	0.13	
BZ4	0.31	0.31	0.31	0.16	0.17	0.17	0.16	0.12	0.14	
Mean	0.31	0.31	0.31	0.16	0.17	0.17	0.16	0.14	0.15	
Z mean	Z0	0.31	0.32	0.31	0.16	0.16	0.16	0.16	0.17	0.16
	Z1	0.31	0.29	0.30	0.16	0.17	0.16	0.17	0.15	0.16
	Z2	0.28	0.31	0.29	0.17	0.18	0.17	0.16	0.15	0.15
	Z3	0.29	0.34	0.31	0.15	0.18	0.16	0.14	0.16	0.15
	Z4	0.28	0.31	0.29	0.14	0.17	0.15	0.16	0.13	0.14
Mean	0.29	0.31		0.15	0.17		0.15	0.15		
LSD _{0.05}	I=ns B=ns Z=ns ZxB=ns IxZ=ns IxB=ns IxBxZ=ns			I=ns B=ns Z=ns ZxB=ns IxZ=ns IxB=ns IxBxZ=ns			I=ns B=ns Z=ns ZxB=ns IxZ=ns IxB=ns IxBxZ=ns			

Table 8

The integrated effect of zeolite and biofertilizer on K/Na, Ca/Na and Mg/Na ratios in bean seeds under different irrigation rates

Amendments	K/Na			Ca/Na			Mg/Na		
	Irr. Rate		Mean	Irr. Rate		Mean	Irr. Rate		Mean
	I ₁	I ₂		I ₁	I ₂		I ₁	I ₂	
Z0	10.9	8.2	9.6	2.35	1.68	2.02	1.38	0.74	1.06
Z1	8.4	10.9	9.7	1.89	2.01	1.95	0.95	1.22	1.09
Z2	8.8	9.8	9.3	1.64	2.00	1.82	1.1	1.24	1.17
Z3	10.1	9.0	9.6	1.93	2.19	2.06	0.93	0.95	0.94
Z4	10.4	11.1	10.8	1.66	2.27	1.97	0.81	1.19	1.00
Mean	9.7	9.8	9.8	1.89	2.03	1.96	1.03	1.07	1.05
BZ0	9.3	12.2	10.8	2.01	2.41	2.21	0.94	1.25	1.10
BZ1	9.0	10.2	9.6	1.79	1.91	1.85	0.93	1.03	0.98
BZ2	10.4	11.2	10.8	1.93	2.21	2.07	1.07	1.15	1.11
BZ3	13.1	12.1	12.6	2.33	2.26	2.30	1.32	1.37	1.35
BZ4	9.8	12.4	11.1	1.96	2.53	2.25	0.98	1.43	1.21
Mean	10.3	11.6	11.0	2.00	2.26	2.13	1.05	1.25	1.15

Amendments	K/Na			Ca/Na			Mg/Na		
	Irr. Rate		Mean	Irr. Rate		Mean	Irr. Rate		Mean
	I ₁	I ₂		I ₁	I ₂		I ₁	I ₂	
Z0	10.1	10.2	10.2	2.18	2.05	2.11	1.16	1.00	1.08
Z1	8.7	10.6	9.6	1.84	1.96	1.90	0.94	1.13	1.03
Z2	9.6	10.5	10.1	1.79	2.11	1.95	1.09	1.20	1.14
Z3	11.6	10.6	11.1	2.13	2.23	2.18	1.13	1.16	1.14
Z4	10.1	11.7	10.9	1.81	2.40	2.11	0.90	1.31	1.10
Mean	10.0	10.7		1.95	2.15		1.04	1.16	
LSD _{0.05}	I=ns B=*0.94 Z=ns ZxB=ns IxZ=ns IxB=ns IxBxZ=ns	Z=ns ZxB=ns IxZ=ns IxB=ns IxBxZ=ns		I=**0.13 B=** 0.10 Z=ns ZxB=ns IxZ=* IxB=ns IxBxZ=*0.40			I=ns B=*0.1 Z=ns ZxB=* IxZ=ns IxB=ns IxBxZ=*0.38		

Addition of biofertilizer led to a significant improvement in mentioned nutrients where their contents were increased in plants treated with low irrigation water amount. As for the interaction between treatments, the highest K/Na ratio was obtained in case of BZ3 x I1, while the highest values of both Ca/Na and Mg/Na were recorded by BZ4 x I2. This may confirm that, under water shortage and salinity stress conditions, the application of biozeolite could be able to improve the quality characteristics of bean seeds by increasing Ca and Mg absorption more than Na.

3.5. Nutrient Contents (kg/fed.)

Irrigation effect was not significant for all studied nutrient contents except for N and Na where, the higher water amount led to increase each of them (Tables 9 and 10).

Phosphorus and Mg content (kg/fed.) trend contradict those obtained by other

nutrients where their contents were increased in plants treated with low irrigation water amount.

Biological fertilization led to increase N, P, K, Ca, Mg and Na content of bean seeds. When focusing on general mean of zeolite and irrespective of control Z0, it can be noticed that, increasing zeolite application rate resulted in a significant increase of all nutrients content following the order: Z4>Z3>Z2>Z1.

In biofertilizer free treatments, the effect of zeolite rates on nutrient contents was different than what could be expected and didn't take consistent trend, whilst, the effect was induced by biozeolite application rate which follows clear direction and ranking in: BZ4>BZ3>BZ2>BZ1. This is logical trend since the same direction was noted with regard to growth traits as previously indicated.

Table 9

The integrated effect of zeolite and biofertilizer on N, P and K content (kg/fed) in bean seeds under different irrigation rates

Amendments	N			P			K		
	Irr. Rate		Mean	Irr. Rate		Mean	Irr. Rate		Mean
	I ₁	I ₂		I ₁	I ₂		I ₁	I ₂	
Z0	26.80	26.60	26.70	0.34	1.68	1.01	9.37	10.03	9.70
Z1	40.10	39.10	39.60	1.78	2.51	2.15	14.72	14.78	14.75
Z2	42.00	38.10	40.05	1.28	2.22	1.75	15.28	15.16	15.22
Z3	53.80	46.80	50.30	3.33	2.72	3.03	22.43	19.49	20.96

Z4	53.50	43.30	48.40	3.54	3.35	3.45	23.90	19.10	21.50	
Mean	43.24	38.78	41.01	2.05	2.50	2.28	17.14	15.71	16.43	
BZ0	53.50	48.10	50.80	2.16	3.04	2.60	20.94	19.41	20.18	
BZ1	38.90	35.90	37.40	2.11	1.60	1.86	14.76	15.17	14.97	
BZ2	42.30	38.90	40.60	3.13	2.17	2.65	16.53	16.20	16.37	
BZ3	48.80	59.90	54.35	1.69	2.76	2.23	20.53	25.90	23.22	
BZ4	81.40	58.80	70.10	3.53	3.49	3.51	31.91	25.06	28.49	
Mean	52.98	48.32	50.65	2.52	2.61	2.57	20.93	20.35	20.64	
Z mean	Z0	40.15	37.35	38.75	1.25	2.36	1.81	15.16	14.72	14.94
	Z1	39.50	37.50	38.50	1.95	2.06	2.00	14.74	14.98	14.86
	Z2	42.15	38.50	40.33	2.21	2.20	2.20	15.91	15.68	15.79
	Z3	51.30	53.35	52.33	2.51	2.74	2.63	21.48	22.70	22.09
	Z4	67.45	51.05	59.25	3.54	3.42	3.48	27.91	22.08	24.99
Mean	48.11	43.55		2.29	2.55		19.04	18.03		
LSD _{0.05}	I=*3.7 B=***3.6 Z=***6.3 ZxB=*** IxZ=* IxB=ns IxBxZ=ns			I=ns B=ns Z=**0.7 ZxB=** IxZ=ns IxB=ns IxBxZ=ns			I=ns B=***1.5 Z=***2.9 ZxB=** IxZ=* IxB=ns IxBxZ=ns			

Table 10

The integrated effect of zeolite and biofertilizer on Ca, Mg and Na content (kg/fed) in bean seeds under different irrigation rates

Amendments	Ca			Mg			Na			
	Irr. Rate		Mean	Irr. Rate		Mean	Irr. Rate		Mean	
	I ₁	I ₂		I ₁	I ₂		I ₁	I ₂		
Z0	2.00	2.11	2.06	1.17	0.94	1.06	0.88	1.28	1.08	
Z1	3.26	2.73	3.00	1.65	1.65	1.65	1.75	1.36	1.56	
Z2	2.87	3.07	2.97	1.92	1.91	1.92	1.75	1.56	1.66	
Z3	4.28	4.78	4.53	2.07	2.06	2.07	2.23	2.21	2.22	
Z4	3.83	3.96	3.90	1.87	2.08	1.98	2.30	1.75	2.03	
Mean	3.25	3.33	3.29	1.74	1.73	1.73	1.78	1.63	1.71	
BZ0	4.51	3.89	4.20	2.13	2.03	2.08	2.39	1.64	2.02	
BZ1	3.12	2.96	3.04	1.53	1.53	1.53	1.75	1.59	1.67	
BZ2	3.12	3.19	3.16	1.72	1.63	1.68	1.62	1.45	1.54	
BZ3	3.69	4.82	4.26	2.07	2.89	2.48	1.61	2.13	1.87	
BZ4	6.58	5.22	5.90	3.27	2.85	3.06	3.39	2.06	2.73	
Mean	4.20	4.02	4.11	2.14	2.19	2.17	2.15	1.77	1.96	
Z mean	Z0	3.26	3.00	3.13	1.65	1.49	1.57	1.64	1.46	1.55
	Z1	3.19	2.85	3.02	1.59	1.59	1.59	1.75	1.48	1.61
	Z2	3.00	3.13	3.06	1.82	1.77	1.80	1.69	1.51	1.60
	Z3	3.99	4.80	4.39	2.07	2.48	2.27	1.92	2.17	2.05
	Z4	5.21	4.59	4.90	2.57	2.47	2.52	2.85	1.91	2.38
Mean	3.73	3.67		1.94	1.96		1.97	1.70		
LSD _{0.05}	I=ns B=***0.4 Z=***0.7 ZxB=** IxZ=ns IxB=ns IxBxZ=ns			I=ns B=**0.2 Z=***0.3 ZxB=** IxZ=ns IxB=ns IxBxZ=ns			I=*0.2 B=*0.3 Z=*0.5 ZxB=* IxZ=* IxB=ns IxBxZ=ns			

3.6. Nutrients Use Efficiency

The intelligent fertilizers are needed to

increase nutrients use efficiency. The values of nitrogen, phosphorus and potassium use efficiency increased in case

of diminishing irrigation water rates; $I_2 > I_1$ (Fig. 5).

Both of zeolite and biofertilization tend to raise nitrogen, phosphorus and potassium use efficiency. The maximum value of nitrogen, phosphorus and potassium use efficiency is associated with the interaction between I_1 x

BZ4, I_2 x BZ3 and I_2 x BZ4. Biozeolite considered as a nutrients reservoir, it increases nutrient retention and provide balanced fertilization, reduces nutrient losses consequently minimizing fertilizer requirements.

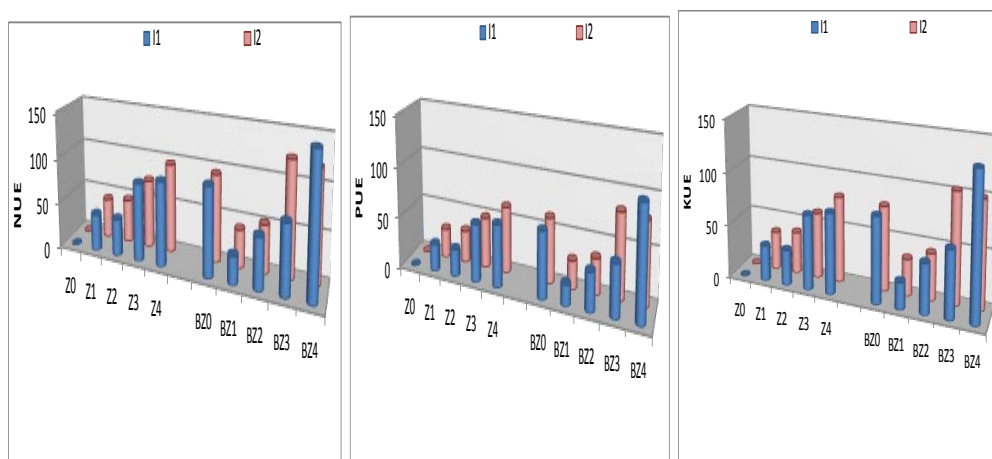


Fig. 5. The integrated effect of zeolite and biofertilizer on NPK use efficiency under different irrigation rates

3.7. Chemical Composition

Total carbohydrate content was reduced in all treatments except I_2 x Z4 as it reached 32.4% compared to control (Z0)

(33.2%). On the other hand, the total carbohydrate content was found to be the highest in biofertilizer treatment I_1 x BZ3 (34.0%) and I_2 x BZ4 (32.0%), respectively (Table 11).

Table 11

The integrated effect of zeolite and biofertilizer on carbohydrate, phenol, tannin and flavonoid under different irrigation rates

Amendments	Carbohydrate			Phenol			Tanin			flavonoid		
	Irr. Rate		Mean	Irr. Rate		Mean	Irr. Rate		Mean	Irr. Rate		Mean
	I ₁	I ₂		I ₁	I ₂		I ₁	I ₂		I ₁	I ₂	
Z0	33.2	20.5	26.9	119.7	101.5	110.6	112.4	98.9	105.7	71.3	46.8	59.1
Z1	22.7	26.9	24.8	137.5	110.1	123.8	91.3	110.6	101.0	59.6	47.4	53.5
Z2	22.0	13.5	17.8	147.0	148.2	147.6	128.5	175.0	151.8	87.0	103.7	95.4
Z3	24.2	25.3	24.8	124.5	115.4	120.0	77.9	114.5	96.2	72.6	155.6	114.1
Z4	21.2	32.4	26.8	97.3	71.0	84.2	92.8	56.2	74.5	41.8	142.7	92.3
Mean	24.7	23.7	24.2	125.2	109.2	117.2	100.6	111.0	105.8	66.5	99.2	82.9
BZ0	18.7	28.4	23.6	129.4	73.8	101.6	77.0	89.7	83.4	35.4	64.1	49.8
BZ1	20.8	16.5	18.7	142.2	82.9	112.6	92.4	45.3	68.9	46.5	75.0	60.8
BZ2	24.0	19.1	21.6	143.7	122.4	133.1	119.8	121.5	120.7	144.5	53.5	99.0
BZ3	34.0	18.3	26.2	240.3	140.8	190.6	207.6	114.1	160.9	51.4	52.7	52.1
BZ4	25.0	32.4	28.7	133.3	73.2	103.3	116.5	76.2	96.4	84.8	193.8	139.3

Amendments	Carbohydrate			Phenol			Tanin			flavonoid			
	Irr. Rate		Mean	Irr. Rate		Mean	Irr. Rate		Mean	Irr. Rate		Mean	
	I ₁	I ₂		I ₁	I ₂		I ₁	I ₂		I ₁	I ₂		
Mean	24.5	22.9	23.7	157.8	98.6	128.2	122.7	89.4	106.0	72.5	87.8	80.2	
Z mean	Z0	26.0	24.5	25.2	124.6	87.7	106.1	94.7	94.3	94.5	53.4	55.5	54.4
	Z1	21.8	21.7	21.7	139.9	96.5	118.2	91.9	78.0	84.9	53.1	61.2	57.1
	Z2	23.0	16.3	19.7	145.4	135.3	140.3	124.2	148.3	136.2	115.8	78.6	97.2
	Z3	29.1	21.8	25.5	182.4	128.1	155.3	142.8	114.3	128.5	62.0	104.2	83.1
	Z4	23.1	32.4	27.8	115.3	72.1	93.7	104.7	66.2	85.4	63.3	168.3	115.8
Mean	24.6	23.3		141.5	103.9		111.6	100.2		69.5	93.5		
LSD _{0.05}	I=0.9 B=ns Z=***1.3 Zx B =*** IxZ=*** Ix B =ns IxBxZ=***2.9			I=***2.9 B =***3.1 Z=***4.1 ZxB=*** IxZ=*** IxB =*** Ix BxZ=***9.1			I=***3.0 B =ns Z=***5.3 ZxB =*** IxZ=*** IxB =*** x BxZ=***9.4			I=***11.7 B =ns Z=***22.1 ZxB=*** IxZ=*** IxB=ns Ix BxZ=***36.9			

Total Phenol, tannin and flavonoid content of the seeds increased with biofertilizer, zeolite interaction under I₁ compared with biofertilizer free treatments.

3.8. Irrigation Water Use Efficiency (IWUE)

In the opposite direction of obtained yield data, irrigation water use efficiency (IWUE) values that were calculated as a relation between bean seed yield and the applied irrigation water seasonally per feddan follow the rank I₂ > I₁ (Fig. 6).

This shows I₂ rate enhanced seed yield

per water unit (m³). Generally, IWUE values ranged between 0.92 and 2.7. Also, IWUE increased with biofertilizer application. Regardless irrigation effect, the relative IWUE values of treated soil with Z₁, Z₂, Z₃, and Z₄ were 48.4, 58.9, 101.1 and 108.4% for biofertilizer free plots and 89.5, 48.4, 50.5, 115.8 and 176.8% for BZ₀, BZ₁, BZ₂, BZ₃ and BZ₄ compared with control, respectively. In absence or present biofertilizer, the highest IWUE values were obtained by I₁ x Z₄ followed by I₂ x Z₄ and the next was I₂ x Z₃ without significant difference between them.

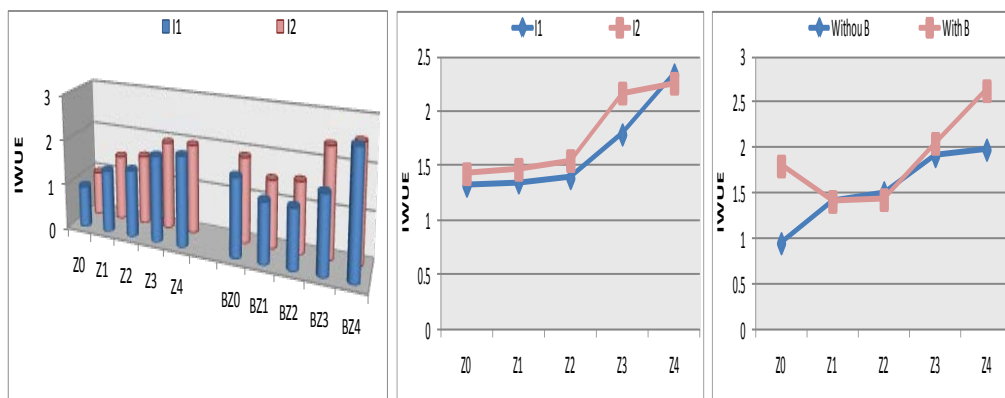


Fig. 6. The integrated effect of zeolite and biofertilizer on IWUE under different irrigation rates

3.9. Economic water productivity (EWP)

The medium of the local market price of bean seeds at 2014/2015 season was evaluated to be 10 EL. This parameter utilized as a part of the assessment of crops

and choice of their appropriateness for cultivation, particularly in the arid and semi-arid region where water shortage is a critical issue. The maximum irrigation water rate (I1) resulted in the minimum EWP value (Fig. 7).

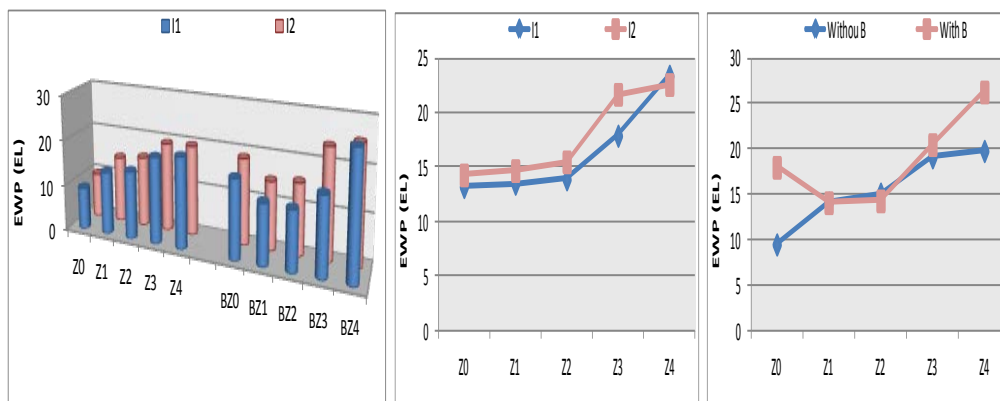


Fig. 7. The integrated effect of zeolite and biofertilizer on EWP under different irrigation rates

Addition of I2 rates increased EWP by 8.5% compared with I1. The productivity of bean seed yield per water unit supplied and its income is higher in I1 x BZ4 treatment followed by I2 x BZ4 treatment and the next is I2 x BZ3 treatment, with few differences between them than that of other treatments. It could be accounted for that the ideal irrigation water administration of bean plants in semi-arid regions is 85% of computed water prerequisite.

4. Discussions

Declining all growth parameters with low water addition is a logical result especially under the studied saline and sandy soil (Table 3) which results in high infiltration rate, high permeability and high water losses, consequently, raising osmotic pressure in root zone with decreasing irrigation water quantity. In another study, plant height, biological, stover and seed

yield of bean plants increased with increasing irrigation water rates from 75 to 100 and 125% of water requirements [2], whereas a negative relationship was detected between salt stress and growth characters [28].

Most plant growth parameters had been significantly enhanced by increasing zeolite addition rates, This finding may be referred to the physical and chemical properties of zeolite that led to 1) reducing soil hardness 2) supplied sufficient water storing and enhancing the water holding capacity of soil, 3) suitable aeration to soil and plant root, 4) decreasing salinity stress. 5) zeolite with a high CEC value causes saving and discharging nutrients easily in the soil and gives the same benefit of the slow release fertilizers (N and K are held by the negatively charged surface and released slowly), 6) reduce washing out of fertilizers to the environment, 7) zeolite improves the cation balance of the plants [6], [10], [12]. Zeolite effect does not rely

upon particle size but depend on the diameter of the internal holes [35].

The application of biofertilizer alone increases the studied growth parameters with high percentage. This may be attributed to, Okadien which contains a group of plant growth promoting bacteria especially rhizobium. Promotion of plant growth using rhizobium can help plant to increasing the growth, increase its protection through enzymes cellulase, protease and lipase productions, enhance plant defense and increase plant ability to tolerating abiotic stresses like salinity and drought [24].

Biozeolite in low rates (BZ1 and BZ2) led to decreasing plant growth parameters values compared to BZ0 and tend to increase by increasing addition rates (BZ3 and BZ4). This may be attributed to I) The three dimensional inorganic structure of zeolite is the stacking of one alumina to five silica tetrahedral and forming an open negatively charged framework with hexagonal void spaces. These net negative charges within the voids hold the nutrients and retain water if zeolite was added solely, this may be occupied by bacteria and discourage its activity when the low rates of mixture were applied. II) A competition between zeolite and bacteria on water and nutrients exists under low rates of zeolite. III) The excessive amount of zeolite in high application rates BZ3 and BZ4 is supplying bacteria with water and nutrients and plays as a benefactor to both plant and bacteria growth. In another study, zeolite at rate 5% produced taller plants, higher biomass and grains of barley than at 1%. This response was perhaps connected with the basic nutrients contained in zeolite in addition to water and nutrient reactions and their dynamics in zeolite treated soils [6]. Biozeolite improves growth and overall crop yield through the retention and timely release of needed nutrients in addition to enhancing

soil physical and chemical. In another study, the application of organic amendments combined with gypsum and zeolite achieved the best result, where it markedly improved soil physical and chemical properties, furthermore it realized the highest net income [25].

On another hand, all nutrient concentrations (%) record an increase in case of low water amount except nitrogen which takes the same trend of yield. This may be due to the dilution effect of high plant growth in I1 treatment. The highest Na% was obtained by control treatment under lowest water quantity (Z0 x I2) and the lowest amount of it was seen in the interactions (BZ3 x I1) and (BZ4 x I2). This may have been partially ascribed to the ability of zeolite to reserve sodium in its void spaces, subsequently, declining Na absorption. Zeolite has ameliorative effects on tomato and rice growth by lessening sodium content in shoots [44]. In the medium contains zeolite, the treated tomato with saline water have high percentages of some macronutrients (K, Ca and Mg) this may be due to zeolite that improves nutrient balance in the medium [10].

The highest K/Na ratio was obtained by BZ3 x I1 and the highest values of both Ca/Na and Mg/Na were recorded by applying BZ4 x I2. This may confirm that, under water shortage and salinity stress conditions, the application of biozeolite could be able to improve the quality characteristics of bean seeds by increasing Ca and Mg absorption more than Na. Zeolite gives alternative calcium to the soil-plant system thus decreasing Na/Ca ratio [6] and Na/K ratios [44]. The provision of Ca from zeolite would prevent the accumulation of toxic Na ion in plants [6]. The different amounts of seeds nutrients approve this subject, where the values of K/Na and Ca/Na in tomato leaves were higher in zeolite treatment [10]. On the other hand, Ghorbani and Babaei [22]

ascribed the amelioration effect of zeolite to adsorbing more potassium ions compared to sodium ions from the solutions resulting in a lower electrical conductivity of potassium containing zeolite compared to sodium.

The high values of nitrogen, phosphorus and potassium use efficiency are associated with the interaction between I2 x BZ3, I2 x BZ4 and I1 x BZ4. The application of biozeolite could have enhanced N, P and K uptake by serving sufficient water in rhizosphere or by developing bean root which is relatively low in case of sole application of zeolite and biofertilizer. Biozeolite is considered as a nutrients reservoir, it increases nutrient retention and provide balanced fertilization, reduces nutrient losses and consequently minimizing fertilizer requirements. The average of nitrogen, phosphorus and potassium use efficiency increased significantly by decreasing water rate, i.e. I3> I2> I1 [30]. Zeolite gradually convey nutritive agrochemically useful nutrients (Na, Ca, K, Mg) to them, ensure a perfect level of cations, and encourage the propagation of useful microorganisms [12]. Zeolite also is widely used to reduce NH₃ volatilization and increase efficiency of N utilization as a slow release nitrogen fertilizer, which in turn to increase nitrogen content and the yield [13].

Carbohydrates are mainly through photosynthesis were lowered in plants exposed to salinity [11]. Most plants are sensitive to salinity that caused a depression in their carbohydrates content [26], [29], by disturbances in the metabolic process leading to increasing the phenolic compounds production [31]. Phenolic compounds are antioxidants against free radicals [27]. Chemical composition of faba bean seeds was noticed by [4] and [20]. Also, Musalam et al. [36] studied the chemical composition of faba bean cultivars under different irrigation

conditions. They showed that ash, protein, and fiber increased under irrigation condition, whereas the opposite was true for fat and carbohydrate.

Values of IWUE were high at most reduced irrigation rate and reduced by increasing water quantity. This trend indicated that low yield produced in water shortage treatment didn't associative with low IWUE, and the development of IWUE values didn't attribute to high irrigation rate. It can be interpreted as follows I) Application of high water rate tends to raise the denominator of IWUE equation (seed yield /total water applied), subsequently decreases the result. II) As for plant nutrition perspective, the plant responses to first application unit, is higher than that after adding second unit. III) The high quantity of irrigation water that may move out of the rhizosphere zone and the plants can't utilize it efficiently. IV) Plants can optimize their water use in short-term and amplify their chance of survival under water stress condition in the long-term [2], [30]. In both cases; absence or presence of biofertilizer, the highest IWUE values were obtained by I1x Z4 followed by I2 x Z4 and the next was I2 x Z3 without any significant difference between them. Thus, it can be anticipated that addition of Z3 or Z4 with the utilization just 85% of water requirement might be produced near values of IWUE, and it can be spared around 15% of applied irrigation water.

There are needs to estimate the economic water productivity established on the expected price before sowing. The enhancement of crop yield per unit of irrigation water quantity does not necessarily result into an expansion in the producers' income because of the non-linearity of yield with the price of outputs [9], but it's important to calculate it especially under dry condition where the irrigation water quantity is a critical factor. Highest irrigation rate has the lowest

economic water productivity. These results are in conformity with the finding in another study which reported that, application of 75% and 100% of water requirements increased EWP of bean by 15.63 and 20.31% compared with excessive water amount, respectively [8]. The top income and bean seed yield for each unit of irrigation water supplied are in I1 x BZ4 followed by I2 x Z4 and the next is I2 x Z3 without a significant difference between them. These few differences refer to biozeolite additions which may be due to rewetting the rhizosphere under water stress treatments.

5. Conclusions

A lack of water supply and salinity are important issues for many countries in arid and semi arid regions. Therefore, finding new intelligent fertilizer to improve water efficiency and economic water productivity in the agricultural sector under abiotic stress conditions is obligatory. A combination between biofertilizers and zeolite is a two-edged sword; both of them can improve or discourage each other; this effect depends on 1) rate of mixing, 2) preparation and preservation methods, 3) time and dose of application, etc. Irrespective of irrigation rates, most studied parameters recorded low values in control treatment compared to treated plots, these reflect the impact of salt stress on growth parameters, yield, nutrient content and chemical composition of bean seeds, as well as confirms that all treated plots could alleviate salinity stress and present high relatively advantage compared with control. Although, the highest bean seed yield was produced by the highest irrigation rate, the quantitative response of most studied parameters to medium irrigation rate in case of using biozeolite at third rate (I2 x BZ3) or fourth

rate (I2 x BZ4) were very close or super than that (I1 x BZ4).

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