



## EFFECT OF BIO-FERTILIZER STRAINS AND ALTERNATIVE SALINE WATER IRRIGATION ON ROSEMARY PLANTS UNDER NORTH SINAI CONDITIONS

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### ABSTRACT

The field experiment was carried out during two successive seasons (2007/2008 and 2008/2009) on rosemary "*Rosmarinus officinalis* L." at El- Shiekh Zowaied Research Station, Desert Research Center, North Sinai Governorate, to study the effect of bio- fertilizers and alternative saline water irrigation on growth, volatile oil yield and chemical constituents of rosemary herb. The plants were treated with *Azotobacter chroococcum* (A), *Bacillus megaterium* (B), the mixture between them (A+B), alternative saline water irrigation [AI(1) 397ppm well water (w.w.) then once of 3117ppm saline water (s.w.), AI(2) 397ppm well water (w.w.) then twice of 3117ppm saline water (s.w.), AI(3) 397ppm (w.w.) then thrice of 3117ppm (s.w)] and the incombination between them. Results showed that, all growth characters; *i.e.*, fresh and dry weights of herb significantly increased with all bio-fertilization treatments compared to control, but significantly decreased with alternative irrigation treatments compared to AI(1). Meanwhile, it showed an increase in yield of herb fresh and dry weights by all interacting treatments. Moreover, all interaction treatments increased significantly the volatile oil yield and also increased carbohydrates and NPK percentages compared to control. Generally, the superior treatment in this connection was that of interaction between (A+B) and AI (1) under North Sinai conditions.

**Key words:** *Rosmarinus officinalis*, bio-fertilizers, alternative irrigation, volatile oil, saline water.

### INTRODUCTION

Rosemary (*Rosmarinus officinalis* L.) plant belongs to family lamiaceae (Labiatae), it is a native of Mediterranean region. Rosemary plant is a shrubby evergreen bush up to 2 meters high with silvery-green, needle, shaped leaves and pole blue flowers (Fawzy, 1985). The whole plant is strongly aromatic and is one of the important medicinal, aromatic and spices plants. It is analgesic, antimicrobial, antioxidant, antiseptic, carminative, fungicidal, nerving, stomachic and tonic. Volatile oil of rosemary constituents are mainly pinene, camphene, limonene, cineole, borneol, camphor, linalool, terpineol, and bornyl acetate (Tamara, 1998).

So, rosemary plant is one of the important plants which need more study in the newly

reclaimed lands especially at North Sinai which has a favorable climatic conditions for producing it. Moreover, studying the agricultural practices and their effect on the herbage and the volatile oil yield must take consideration.

Bio-fertilization is one of the important factors used to produce products free from mineral contamination. On the other hand, the intensive use of chemical fertilizers caused environmental pollution problems and high rates of it decrease the potential activity of micro flora and the stability of organic matter. Hence, the attention had been focused on the researches of bio-fertilizers to substitute chemical fertilizers. Bio-fertilizers increase the number of micro-organisms and accelerate certain microbial processes in the rhizosphere of inoculated soils or plants which can change the

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unavailable forms of nutrients into available ones (Subb Roa, 1981; Alaa El-Din, 1982). Generally, bio-fertilizers increased significantly vegetative growth, *i.e.*; fresh and dry weights / plant (Hashem, 2007 on thyme; Al-Fraihat *et al.*, 2011 on marjoram; Abdullah *et al.*, 2012 on rosemary; Darzi and Seyed-Hadi, 2012 on coriander). Moreover, the volatile oil yield was increased with application of bio-fertilizers as reported by Hashem (2007) on thyme, Al-Fraihat *et al.* (2011) on marjoram plants. and Darzi (2012) on anise.

The salinization of Egyptian soils rapidly going to be an acute problem. Moreover, increased need for salt tolerant medicinal and aromatic plants is still continuous due to both increased restriction of water resources and saline water intrusion into ground water, especially in newly reclaimed lands. Under salinity condition, the most problem facing agricultural production in irrigated arid and semi arid areas is how to prepare a suitable root zone. Salts inhibit plant growth and development by increasing the osmotic stress, specific ion toxicity and nutrient imbalance. The extent of damage depends on severity of stress, growth condition and plant sensitivity to salinity (Cornillon and Palliox, 1997).

The reduction in herb fresh and dry weights / plant by using saline water for irrigation was also found by Zaki *et al.* (2009) on sweet fennel, Ghanavat and Sengul (2010) on *Chamomilla recutita*, *Matricaria recutita* and *Matricaria chamomilla* plants and Khadhri *et al.* (2012) on *Cymbopogon schoenanthus*. Moreover, the decrease in oil yield by using saline water for irrigation was also obtained by Ismail (2005) on fennel, Aziz *et al.* (2008) on peppermint, pennyroyal, and apple mint plants and Zaki *et al.* (2009) on sweet fennel. Also, Mahmoud and Mohamed (2008) on wheat, Leithy *et al.* (2009) on geranium and El-Rys (2012) on wheat found that using bio-fertilizers under saline conditions overcame the harmful effects of salinity on plant and increased the yield.

The present study aimed to investigate the influence of diazotrophs and phosphorus dissolving bacteria and alternative saline water for irrigation on growth, volatile oil and chemical constituents of rosemary plants.

## MATERIALS AND METHODS

The present work was carried out at the Experimental Station of Desert Research Center (D.R.C.) at El-Sheikh Zowayed, North Sinai Governorate during the two successive seasons of 2007/2008 and 2008/2009.

Seedlings of rosemary (*Rosmarinus officinalis*) plants were kindly provided from the experimental station of (D.R.C.) at El-Sheikh Zowayed. The Seedlings were planted in the nursery bed on 15<sup>th</sup> October in the two seasons. Meanwhile, seedlings were transplanted in the experimental area on 15<sup>th</sup> February (2008 and 2009) for two seasons in sandy soil. The mechanical and chemical properties of the used soil are shown in Table A.

The irrigation system of the experiment was drip irrigation with the drippers of four liters/h for one hour twice every week by using plastic tanks on the first of lateral side. The lateral sides were pipe lines from plastic material diameter 16 mm and with 41.4 m tall. The spaces between them (pipe) were 75 cm, 30 cm between the plants on the row and 2 m between the treatments. The lateral side of every replicate was 8.4 m and contained 28 plants. Every treatment had three replicates and contained 84 plants. The chemical analysis of the used water is shown in Table B.

### Bio-Fertilization Treatments (BF)

The biological fertilization strains were obtained from Microbiology Department, Desert Research Center (D.R.C.) and were as follows.

- *Azotobacter chroococcum* (A), nitrogen fixing bacteria with rate of  $10^8$  cells/ml in aqueous suspension.
- *Bacillus megaterium* (B), phosphate dissolving bacteria with rate of  $10^8$  cells/ml,
- The mixture of the two mentioned strains (A+B) with rate of  $10^8$  cells/ml with equal volume. Every treatment had three replicates, every one received 100 ml of aqueous suspension diluted with 10 litres of tap water, and the control plants were treated with tap water.

These strains were added in the root zone twice every season; *i.e.*, after 15 days from transplanting and after the first cut (after 120 days from transplanting date).

**Table A. Mechanical and chemical properties of the used soil**

Characters	Mechanical analysis					Chemical analysis				
	Fine sand (%)	Coarse sand (%)	Silt (%)	Clay (%)	Texture class	pH	E.C. (mmhos / cm)	Organic matter (%)	Available N (ppm)	Available P (ppm)
Values	29.25	56.78	10.52	3.45	Sandy	8.20	0.95	0.90	10.13	3.75

**Table B. Chemical analysis of irrigation water used**

No.	EC (mmhos / cm)	pH	Soluble cations (ppm)				Soluble anions (ppm)			
			Ca <sup>++</sup>	Mg <sup>++</sup>	Na <sup>+</sup>	K <sup>+</sup>	CO <sub>3</sub> <sup>-</sup>	HCO <sub>3</sub> <sup>-</sup>	SO <sub>4</sub> <sup>-</sup>	Cl <sup>-</sup>
W1	0.620	8.3	41.20	24.92	55.00	2.00	23.22	86.55	89.00	85.50
W2	4.870	7.4	147.60	154.70	650.00	6.00	11.61	157.38	770.00	973.75

### Alternative Saline Water Irrigation Treatments (AI)

- AI (1): Irrigation with 397 ppm well water (w.w.) which was recommended in the region then once of 3117 ppm saline water (s. w.) as a control,
- AI(2): Irrigation with 397 ppm well water (w.w.) then twice of 3117 ppm saline water (s.w.), and
- AI(3): Irrigation with 397 ppm well water (w.w.) then thrice of 3117 ppm saline water (s.w.).

### Interaction Treatments Between Bio-Fertilization Strains (BF) and Alternative Saline Water Irrigation (AI)

Each bio-fertilization strain (four strains including control) combined with each treatment of Alternative saline water irrigation treatments (three treatments) to form 12 interaction treatments.

### Design and Statistical Analysis

The experimental design was factorial experiment between bio-fertilization strains and alternative irrigation in split plot design, the main plots were the alternative saline water irrigation and the subplots were bio-fertilization strains. The experiment included three replicates, each replicate contained 28 plants. Data of the present study were statistically analyzed and the differences between the means of the treatments were considered significant

when they were more than least significant differences (L.S.D.) at the 5% or 1% levels according to Steel and Torrie (1980).

### Harvesting

Harvesting of herb was carried out in two cuts every season. The first cut was on 15<sup>th</sup> June (after 120 days from transplanting date) and the second cut was on 15<sup>th</sup> October (after 240 days from transplanting date) by cutting the vegetative parts of plants (5 cm above the soil surface) leaving 2 branches for regrowth. All the plants received normal agricultural practices when they needed.

A random sample of four plants from each treatment was taken before the first cut, on 14<sup>th</sup> June also the second cut on 14<sup>th</sup> October and the following data were recorded:

### Vegetative characters

- Herb fresh and dry weights / plant (g).

### Volatile oil production

- Volatile oil yield / plant and fad. (ml) according to British Pharmacopoeia (1963).

### Determination of some chemical constituents

- Total carbohydrates, nitrogen, phosphorus and potassium percentages were determined in dried harvested leaves of rosemary plants at 70°C for 72 hours according to AOAC (1980).

## RESULTS AND DISCUSSIONS

### Effect of Bio-Fertilization Strains (BF) and Alternative Saline Water Irrigation (AI) as well as their Interaction Treatments on Vegetative Growth

#### Herb fresh and dry weights / plant (g)

##### Effect of bio-fertilization (BF)

As regard to herb fresh and dry weights per plant, it could be noticed from the data presented in Table 1 that, herb fresh and dry weights per plant gradually increased by using the treatments of *Bacillus* (B) followed by *Azotobacter* (A) and then (A+B). Moreover, the best treatment was the addition of (A+B) which gave the highest value in this respect and showed significant increase compared to other ones under study. Since, the addition of (A), (B) or (A+B) individually led to highly significant increase in this regard compared to control. These results were similar in both cuts of the two seasons. These results are similar to those found by Hashem (2007) on thyme, Al-Fraihat *et al.* (2011) on marjoram, Abdullah *et al.* (2012) on rosemary and Darzi and Seyed-Hadi (2012) on Coriander.

The increase in plant fresh and dry weights by using bio-fertilization may be due to the increase of N uptake in the root zone as a result of N fixation by bacteria. Also, the solubilization of mineral nutrients, synthesis of vitamins, amino acids and gibberellins as a result of bio-fertilization might stimulate growth and yield, Sprent (1990). Furthermore, the ability of *Azotobacter* to produce growth and antifungal substances in addition to the nitrogen fixation which became available to plants and probably the reason of higher yields, Mishutin and Shilnikova (1971).

##### Effect of alternative saline water irrigation (AI)

The data given in Table 1 suggest that the treatment of [397ppm (w.w.) then thrice of 3117ppm (s.w.) AI(3)] gave a highly significant decrease in herb fresh and dry weights per plant compared to those of [397ppm well water (w.w.) then twice of 3117ppm saline water (s.w.) AI(2)] and [397ppm (w.w.) then once of 3117ppm (s.w.) AI(1)]. While, irrigation rosemary plants with AI(2) or AI(3) gave a highly significant

decrease in herb fresh and dry weights per plant compared to AI(1) treatment. These results hold true in the two cuts of the two seasons. Similar results were stated by Abd El- Wahab (2006) on fennel plant. Moreover, the decrease in herb fresh and dry weights per plant by using saline water irrigation was also recorded by Zaki *et al.* (2009) on sweet fennel, Leithy *et al.* (2009) on geranium, Ghanavat and Sengul (2010) on *Chamomilla recutita*, *Matricaria recutita* and *Matricaria chamomilla* plants and Khadhri *et al.* (2012) on *Cymbopogon schoenanthus*.

Such decrease in fresh and dry weights might be due to that salinity increased osmotic pressure which caused a drop in plant water content as found by Sanchezconde and Azura (1979) on tomato plants. High salinity levels could cause a depression in photosynthetic activities resulting in low CO<sub>2</sub> fixation. The absorption of minerals could be retarded leading to low plant metabolism.

##### Effect of interaction treatments between (BF) and (AI)

As shown in Table 1, the results reveal that the interaction treatment between (A+B) and AI(1) recorded the highest herb fresh and dry weights per plant and gave highly significant increase in this respect compared to the other interaction ones. In addition, the interaction treatments between alternative saline water irrigation [AI(1), AI(2) or AI(3)] with biological strains (B), (A) and (A+B) gave insignificant increase in herb fresh and dry weights per plant in the first cut of first season, but in the second cut it recorded a highly significant increase in this regard compared to the treatment of uninoculated with alternative saline water irrigation in both seasons. These results are in harmony with those found by Leithy *et al.* (2009) on geranium. In this respect, Mahmoud and Mohamed (2008), Swaefy and Basuny (2011) on *Wedelia trilobata*, Khodair *et al.* (2008) and El-Rys (2012) on wheat found that bio-fertilization decreased the harmful effect of salinity and increased total yield

Generally, bio-fertilization reduced, to some extent, the harmful of water salinity up to AI(3) on herb fresh and dry weights as found in this work, which might be due to their enhancing effects on the vegetative growth, throughout the increase in metabolites.

**Table 1. Effect of bio-fertilization strains (BF), alternative saline water irrigation (AI) and their interaction treatments on herb fresh and dry weights / plant (g) of rosemary plants of two cuts during two seasons**

Alternative irrigation (AI)	Herb fresh weight / plant (g)								Herb dry weight / plant (g)							
	AI(1)	AI(2)	AI(3)	$\bar{X}$ (BF)	AI(1)	AI(2)	AI(3)	$\bar{X}$ (BF)	AI(1)	AI(2)	AI(3)	$\bar{X}$ (BF)	AI(1)	AI(2)	AI(3)	$\bar{X}$ (BF)
	First season				Second season				First season				Second season			
	Bio Fertilization(BF)															
	<b>First cut</b>															
Control	44.58	36.67	30.08	37.11	63.23	54.25	40.33	52.61	10.70	8.93	7.22	8.95	15.48	13.06	10.00	12.85
Bacillus (B)	56.25	55.83	48.33	53.47	80.33	77.37	69.20	75.63	14.06	13.93	12.08	13.36	19.20	18.15	16.61	17.98
Azotobacter (A)	67.50	62.92	58.33	67.22	99.28	91.23	87.18	92.57	17.55	16.36	14.41	16.11	23.10	21.68	20.10	21.62
(A + B)	97.50	82.92	71.25	83.89	145.98	127.03	105.17	126.06	26.33	22.91	19.24	22.83	33.12	30.01	24.19	29.10
$\bar{X}$ (AI)	66.46	59.59	52.00		97.21	87.47	75.47		17.16	15.53	13.24		22.72	20.72	17.72	
L.S.D. at 5%For	(BF)=4.91	(AI)=3.75	(BF)x(AI)=N.S.		(BF)=1.02	(AI)=0.84	(BF)x(AI)=1.76		(BF)=4.17	(AI)=1.75	(BF)x(AI)=N.S.		(BF)=0.48	(AI)=0.36	(BF)x(AI)=0.84	
L.S.D. at 1%For	(BF)=6.73	(AI)=6.22	(BF)x(AI)=N.S.		(BF)=1.39	(AI)=1.39	(BF)x(AI)=2.41		(BF)=5.71	(AI)=2.91	(BF)x(AI)=N.S.		(BF)=0.66	(AI)=0.60	(BF)x(AI)=1.15	
	<b>Second cut</b>															
Control	52.08	41.67	27.83	40.53	57.67	50.64	41.03	49.78	12.50	10.28	6.68	9.82	14.41	12.91	10.26	12.53
Bacillus (B)	77.92	70.00	63.75	70.56	105.00	89.20	87.28	93.83	19.48	17.50	15.94	17.64	25.20	21.41	20.95	22.52
Azotobacter (A)	109.58	100.00	89.17	99.58	142.70	128.65	119.35	130.23	28.16	26.00	23.19	25.73	33.38	30.19	27.89	30.49
(A + B)	202.50	161.25	130.83	164.86	244.32	208.05	169.42	207.26	54.68	43.54	35.33	44.52	56.20	48.30	38.97	47.82
$\bar{X}$ (AI)	110.52	93.23	77.90		137.42	119.14	104.27		28.71	24.33	20.29		32.30	28.20	24.52	
L.S.D. at 5%For	(BF)=7.42	(AI)=9.16	(BF)x(AI)=12.90		(BF)=1.30	(AI)=0.95	(BF)x(AI)=2.26		(BF)=2.00	(AI)=2.40	(BF)x(AI)=N.S.		(BF)=0.45	(AI)=0.31	(BF)x(AI)=0.79	
L.S.D. at 1%For	(BF)=10.17	(AI)=15.19	(BF)x(AI)=17.61		(BF)=1.78	(AI)=1.58	(BF)x(AI)=3.09		(BF)=2.75	(AI)=3.99	(BF)x(AI)=N.S.		(BF)=0.62	(AI)=0.51	(BF)x(AI)=1.08	

AI(1)=397ppm well water ( w. w.) then once of 3117ppm saline water ( s.w.) AI(2)= 397ppm ( w. w.) then twice of 3117ppm ( s.w.)

AI(3)= 397ppm ( w. w.) then thrice of 3117ppm ( s.w.)

### **Effect of Bio-Fertilization Strains (BF) and Alternative Saline Water Irrigation (AI) as well as their Interaction Treatments on Volatile Oil Production of Rosemary Plant**

#### **Volatile oil yield per plant and faddan (ml)**

##### **Effect of bio-fertilization strains (BF)**

The data presented in Table 2 show that volatile oil yield per plant and fad., were gradually increased by using (B) followed by (A) and (A+B) strain treatments. In the same time, all bio-fertilization strains (B), (A) and (A+B) treatments led to a highly significant increase in this respect compared to control. Also, the highest value of volatile oil yield per plant and fad., resulted from (A+B) treatment and showed highly significant increase in this regard compared to (A), (B) and control treatments. These results were recorded in both cuts of the two seasons. These results are in accordance with those reported by Hashem (2007) on thyme, Pablo *et al.* (2008) on *Origanum majorana*, Al-Fraihat *et al.* (2011) on marjoram and Darzi (2012) on anise plants.

The stimulatory effect of the treatments of bio-fertilization on volatile oil yield may be due to the positive role of bio-fertilization on plant growth. In addition, inoculation with a symbiotic nitrogen fixers and phosphate dissolving bacteria may led to the increase of nitrogen and phosphorus in plant tissues at a considerable rate to build up more metabolites necessary for inducing the volatile oil synthesis.

##### **Effect of alternative saline water irrigation (AI)**

The obtained data in Table 2 indicate that there was significant decrease in volatile oil yield per plant and fad., by using AI (2) and AI(3) treatments. Also, the highest value in volatile oil yield per plant and fad., were obtained from AI(1) treatment, then decreased gradually with AI(2) followed by AI(3) treatments. These results are in harmony with those respected by Abd El- Wahab (2006) on fennel plants. The reduction in volatile oil yield per plant by using saline water for irrigation was also observed by Ismail (2005) on fennel, Aziz *et al.* (2008) on peppermint, pennyroyal, and apple mint plants and Zaki *et al.* (2009) on sweet fennel.

However, the reduction in oil yield due to salinity treatments could be mainly due to the

decrease in plant herb weight and growth, as found in the present study. In this regard, Penka (1978) showed that the formation and accumulation of essential oils in plants was explained as due to the action of environmental factors. It might be claimed that the formation and accumulation of essential oil was directly depended upon perfect growth and development of the plants production oils. Moreover, the decrease in oil production might be due to the decrease in plant anabolism.

##### **Effect of interaction treatments between (BF) and (AI)**

The data given in Table 2 demonstrate that, the interaction treatment between (A+B) and AI(1) recorded highly significant increase in volatile oil yield per plant and fad., and gave the maximum value in this regard compared to other interaction ones and control. In the meantime, the interaction treatments between all bio-fertilization strains (B), (A) and (A+B) with alternative irrigation [AI(1), AI(2) or AI(3)] recorded significant increase in this respect compared to control. These results were found in the two cuts during the two seasons. In this concern Hashem (2007) on thyme, Pablo *et al.* (2008) on *Origanum majorana*, Al-Fraihat *et al.* (2011) on marjoram and Darzi (2012) on anise plants mentioned that bio-fertilization treatments increased volatile oil yield. However, such results might be due to that bio-fertilization treatments showed similar effect in this respect in the two cuts of the two seasons as mentioned just before. Also, Ismail (2005) on fennel plants found that using saline water irrigation decreased volatile oil yield. However, such results might be due to that saline water irrigation treatments showed similar effect in this respect in the two cuts of the two seasons as mentioned.

### **Effect of Bio-Fertilization Strains (BF) and Alternative Saline Water Irrigation (AI) as well as their Interaction Treatments on Chemical Constituents of Rosemary Plant**

#### **Total carbohydrates, nitrogen, phosphorus and potassium percentages (%)**

##### **Effect of bio-fertilization strains (BF)**

The data given in Tables 3 and 4 clear that, the highest total carbohydrates, nitrogen, phosphorus and potassium percentages resulted

**Table 2. Effect of bio-fertilization strains (BF), alternative saline water irrigation (AI) and their interaction treatments on volatile oil yield / plant and fad., (ml) of rosemary plants of two cuts during two seasons**

Bio Fertilization(BF)	Alternativ irrigation (AI)		Volatile oil yield / plant (ml)						Volatile oil yield / fed (ml)							
	AI(1)	AI(2)	AI(3)	$\bar{X}$ (BF)	AI(1)	AI(2)	AI(3)	$\bar{X}$ (BF)	AI(1)	AI(2)	AI(3)	X (BF)	AI(1)	AI(2)	AI(3)	$\bar{X}$ (BF)
	First season			Second season			First season			Second season						
	First cut															
Control	0.0199	0.0183	0.0155	0.0180	0.0313	0.0290	0.0244	0.0282	358.2	330.0	279.0	322.4	563.4	522.0	439.2	508.2
Bacillus (B)	0.0333	0.0300	0.0240	0.0291	0.0497	0.0423	0.0393	0.0438	600.0	540.0	432.0	524.0	894.0	762.0	708.0	788.0
Azotobacter (A)	0.0443	0.0380	0.0310	0.0378	0.0633	0.0540	0.0533	0.0569	798.0	684.0	558.0	680.0	1140.0	972.0	960.0	1024.0
(A + B)	0.0740	0.0610	0.0457	0.0602	0.1017	0.0843	0.0657	0.0839	1332.0	1098.0	822.0	1084.0	1830.0	1518.0	1182.0	1510.0
$\bar{X}$ (AI)	0.0429	0.0368	0.0291		0.0615	0.0524	0.0457		772.0	663.0	522.8		1107.0	943.5	822.3	
L.S.D. at 5%For	(BF)=0.0030	(AI)=0.0038	(BF)x(AI)=0.0059		(BF)=0.0014	(AI)=0.0017	(BF)x(AI)=0.0025		(BF)=55.0	(AI)=69.4	(BF)x(AI)=106.9		(BF)=25.8	(AI)=30.2	(BF)x(AI)=44.8	
L.S.D. at 1%For	(BF)=0.0042	(AI)=0.0063	(BF)x(AI)=0.0087		(BF)=0.0020	(AI)=0.0028	(BF)x(AI)=0.0034		(BF)=75.4	(AI)=115.2	(BF)x(AI)=157.2		(BF)=35.4	(AI)=50.1	(BF)x(AI)=61.3	
	Second cut															
Control	0.0321	0.0277	0.0197	0.0265	0.0313	0.0300	0.0248	0.0290	578.0	498.0	355.0	477.0	563.4	552.0	446.4	520.6
Bacillus (B)	0.0607	0.0510	0.0450	0.0522	0.0667	0.0530	0.0523	0.0573	1092.0	918.0	810.0	940.0	1200.0	954.0	942.0	1032.0
Azotobacter (A)	0.0923	0.0777	0.0653	0.0784	0.0953	0.0823	0.0760	0.0846	1662.0	1398.0	1176.0	1412.0	1716.0	1482.0	1368.0	1522.0
(A + B)	0.1853	0.1327	0.1047	0.1409	0.1777	0.1453	0.1150	0.1460	3336.0	2388.0	1884.0	2536.0	3198.0	2616.0	2070.0	2628.0
$\bar{X}$ (AI)	0.0926	0.0722	0.0586		0.0928	0.0778	0.0670		1667.0	1300.5	1056.3		1669.4	1401.0	1206.6	
L.S.D. at 5%For	(BF)=0.0074	(AI)=0.0113	(BF)x(AI)=0.0158		(BF)=0.0023	(AI)=0.0011	(BF)x(AI)=0.0039		(BF)=134.8	(AI)=203.7	(BF)x(AI)=284.3		(BF)=41.1	(AI)=20.7	(BF)x(AI)=71.18	
L.S.D. at 1%For	(BF)=0.0103	(AI)=0.0188	(BF)x(AI)=0.0236		(BF)=0.0031	(AI)=0.0019	(BF)x(AI)=0.0054		(BF)=184.7	(AI)=337.7	(BF)x(AI)=425.1		(BF)=56.3	(AI)=34.3	(BF)x(AI)=97.52	

AI(1)=397ppm well water ( w. w.) then once of 3117ppm saline water ( s.w.) AI(2)= 397ppm ( w. w.) then twice of 3117ppm ( s.w.)

AI(3)= 397ppm ( w. w.) then thrice of 3117ppm ( s.w.)

**Table 3. Effect of bio-fertilization strains (BF), alternative saline water irrigation (AI) and their interaction treatments on total carbohydrates and nitrogen percentages of rosemary leaves of two cuts during two seasons**

Alternative irrigation (AI)	Total carbohydrates percentage								Total nitrogen percentage							
	AI(1)	AI(2)	AI(3)	$\bar{X}$ (BF)	AI(1)	AI(2)	AI(3)	$\bar{X}$ (BF)	AI(1)	AI(2)	AI(3)	$\bar{X}$ (BF)	AI(1)	AI(2)	AI(3)	$\bar{X}$ (BF)
	First season				Second season				First season				Second season			
<b>Bio Fertilization(BF)</b>																
									First cut							
Control	3.750	3.745	3.644	3.713	4.422	3.950	3.788	4.053	1.91	1.86	1.77	1.85	3.26	3.01	2.87	3.05
Bacillus (B)	5.084	4.074	3.812	4.323	4.723	4.525	3.994	4.414	2.30	1.98	1.96	2.08	3.88	3.39	3.34	3.54
Azotobacter (A)	5.206	4.090	4.057	4.451	5.210	4.726	4.696	4.877	2.41	2.31	2.11	2.28	4.71	3.99	3.53	4.08
(A + B)	5.368	5.321	4.655	5.115	5.527	5.378	5.281	5.395	2.60	2.51	2.12	2.41	5.71	4.58	3.79	4.69
$\bar{X}$ (AI)	4.852	4.308	4.042		4.971	4.645	4.440		2.31	2.17	1.99		4.39	3.74	3.38	
									Second cut							
Control	4.968	4.915	4.741	4.875	5.123	5.122	4.990	5.078	2.90	2.88	2.50	2.76	3.60	3.55	3.07	3.41
Bacillus (B)	5.173	4.988	4.958	5.040	5.982	5.639	5.607	5.743	3.85	3.69	3.03	3.52	4.61	4.40	3.73	4.25
Azotobacter (A)	5.437	5.350	5.057	5.400	6.287	6.051	5.772	6.037	4.12	3.98	3.15	3.75	5.15	4.82	3.98	4.65
(A + B)	5.700	5.533	5.412	5.548	6.308	6.123	5.965	6.132	4.89	4.64	3.90	4.48	6.30	5.57	4.74	5.54
$\bar{X}$ (AI)	5.320	5.196	5.042		5.925	5.733	5.583		3.94	3.80	3.15		4.92	4.59	3.88	

AI(1)=397ppm well water ( w. w.) then once of 3117ppm saline water ( s.w.) AI(2)= 397ppm ( w. w.) then twice of 3117ppm ( s.w.) AI(3)= 397ppm ( w. w.) then thrice of 3117ppm ( s.w.) .

**Table 4. Effect of bio-fertilization strains (BF), alternative saline water irrigation (AI) and their interaction treatments on total phosphorus and potassium percentages of rosemary leaves of two cuts during two seasons**

Alternative irrigation(AI)	Total phosphorus percentage								Potassium percentage							
	AI(1)	AI(2)	AI(3)	$\bar{X}$ (BF)	AI(1)	AI(2)	AI(3)	$\bar{X}$ (BF)	AI(1)	AI(2)	AI(3)	$\bar{X}$ (BF)	AI(1)	AI(2)	AI(3)	$\bar{X}$ (BF)
	First season				Second season				First season				Second season			
<b>Bio Fertilization(BF)</b>																
									First cut							
Control	0.600	0.565	0.539	0.568	0.510	0.490	0.460	0.490	1.435	1.410	1.280	1.375	2.271	2.235	2.124	2.210
Bacillus (B)	0.689	0.680	0.609	0.659	0.590	0.580	0.520	0.570	1.533	1.410	1.353	1.432	2.394	2.267	2.214	2.292
Azotobacter (A)	0.803	0.786	0.645	0.745	0.720	0.640	0.570	0.640	1.607	1.575	1.386	1.523	2.452	2.417	2.312	2.394
(A + B)	0.874	0.812	0.653	0.780	0.770	0.740	0.580	0.700	1.648	1.624	1.510	1.594	2.494	2.462	2.403	2.453
$\bar{X}$ (AI)	0.742	0.711	0.612		0.650	0.610	0.530		1.556	1.505	1.382		2.403	2.345	2.263	
									Second cut							
Control	0.698	0.618	0.565	0.627	0.650	0.600	0.560	0.600	2.747	2.755	2.657	2.720	3.186	3.049	3.104	3.113
Bacillus (B)	0.724	0.671	0.662	0.686	0.700	0.660	0.640	0.670	2.829	2.772	2.681	2.761	3.383	3.286	3.210	3.293
Azotobacter (A)	0.795	0.759	0.680	0.745	0.800	0.740	0.690	0.750	2.936	2.878	2.780	2.865	3.481	3.341	3.415	3.412
(A + B)	0.848	0.804	0.742	0.798	1.020	0.780	0.740	0.850	3.075	3.009	2.878	2.987	3.674	3.527	3.424	3.542
$\bar{X}$ (AI)	0.766	0.713	0.662		0.790	0.700	0.660		2.897	2.853	2.749		3.431	3.301	3.288	

AI(1)=397ppm well water ( w. w.) then once of 3117ppm saline water (s.w.) AI(2)= 397ppm ( w. w.) then twice of 3117ppm ( s.w.) AI(3)= 397ppm ( w. w.) then thrice of 3117ppm ( s.w.) .



from (A+B) treatment compared to (B), (A) and control. Moreover, the treated plants with *Azotobacter* (A), *Bacillus* (B) and the mixture between them (A+B) individually recorded an increase in this concern compared to control. These results were found in the two cuts of the two seasons. Similar results were stated by Hashem (2007) on thyme, Hassan (2009) on *Hibiscus sabdariffa* and Hellal *et al.* (2011) on dill plant.

The increment in total carbohydrates may be due to the increase of photosynthesis as a result of the increase in photosynthesis pigment content in the leaves, (Tyler *et al.*, 1988). Furthermore, the increase in nitrogen percentage may be due to bio-fertilization by fixing nitrogen bacteria which utilized as a source of nitrogen. The application of bio-fertilization increased the concentration of N in plant tissues and also the total fresh herbage yield, which ultimately led to the increase of N uptake. Moreover, the increment of phosphorus percentage in plant tissues as a result of bacteria which secrete organic acids lead to transferring fixed phosphate to available phosphate, causing the increment of available phosphorus in the root zone, which increase P uptake (Burger *et al.*, 1997). Also, the increase in NPK percentages might be due to the direct or indirect effect of the used bio-fertilization treatments on the absorption and / or translocation of NPK in the plants.

#### **Effect of alternative saline water irrigation (AI)**

From data recorded in Tables 3 and 4, it is obvious that, the alternative irrigation treatments AI(2) or AI(3) exhibited a decrease in total carbohydrates, nitrogen, phosphorus and potassium percentages compared to AI(1). The highest values in this regard were obtained from AI(1) treatment, then decreased gradually with AI(2) followed by AI(3) treatments. These results took the same trend in both cuts of the two seasons. Such results are in agreement with those obtained by Abd El- Wahab (2006). Also, the decrease in carbohydrates and NPK percentages by using saline irrigation water was also found by Ismail (2005); Abou El-Magd *et al.* (2008) and Zaki *et al.* (2009) on fennel.

In this concern, Kabanov *et al.* (1973) on pea mentioned that high salinity levels caused a depression of photosynthetic activities, resulting in low CO<sub>2</sub> fixation. The absorption of minerals could be retarded leading to low plant metabolism. Moreover, Strogonov (1962) suggested that, under high level of chloride salinity, the accumulation of nitrogen being much more rapid than its utilization in the physiological processes and formation of new cell tissues. Furthermore, Ashour *et al.* (1970) reported that chloride salinity retarded translocation of P<sup>32</sup> from root towards the above ground parts of sunflower plants. Meanwhile, the decrease in NPK percentages might be due to irrigation with saline water which led to a decrease in absorption and/or translocation of NPK.

#### **Effect of interaction treatments between (BF) and (AI)**

From data presented in Tables 3 and 4, it is clear that the interaction between (A+B) and AI(1) was superior treatment in increasing total carbohydrates, nitrogen, phosphorus and potassium percentages which gave the highest values in this regard if compared with other interaction treatments. Furthermore, the interaction treatments between biological strains (B), (A) and (A+B) with alternative irrigation AI(1), AI(2) or AI(3) showed an increase in total carbohydrates and NPK percentages compared with control. These results were similar in the first and second cuts of the two seasons. In this respect, Zuccarini (2007) on lettuce found that bio-fertilizer stimulated the absorption of K under salinity conditions. Such increase in nitrogen, phosphorus and potassium percentages caused by using the interaction treatments might be due to that the used bio-fertilization treatments might enhance NPK absorption and/or translocation from the roots to the above-ground parts under salinity conditions.

Generally, these results bear indication that the used bio-fertilization could overcome the harmful effects of salinity on carbohydrates and NPK percentages of rosemary herb.

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## تأثير التسميد الحيوي والري التبادلي على نبات الحاصلبان تحت ظروف شمال سيناء

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أجريت تجربة حقلية في موسمين متتابعين (٢٠٠٧/٢٠٠٨ و ٢٠٠٨/٢٠٠٩) على نبات الحاصلبان (*Rosmarinus officinalis L.*) في محطة بحوث شمال سيناء بالشيخ زايد التابعة لمركز بحوث الصحراء بغرض دراسة تأثير السماد الحيوي والري التبادلي على النمو ومحصول الزيت الطيار والمكونات الفعالة (الكربوهيدرات والنيتروجين والفوسفور والبوتاسيوم)، وقد كانت المعاملات هي: استخدام السماد الحيوي من البكتريا المثبتة للنترجين (*Azotobacter chroococcum* (A) ، والبكتريا المذيبة للفوسفور (*Bacillus megaterium* (B) ، والخليط بينهما (A+B)، والري التبادلي " الري مرة واحدة بماء ملوحتة ٣٩٧ جزء في المليون يعقبه الري مرة واحدة بماء ملوحتة ٣١١٧ جزء في المليون (AI(1) والري مرة واحدة بماء ملوحتة ٣٩٧ جزء في المليون يعقبه الري مرتان بماء ملوحتة ٣١١٧ جزء في المليون (AI(2) والري مرة واحدة بماء ملوحتة ٣٩٧ جزء في المليون يعقبه الري ثلاث مرات بماء ملوحتة ٣١١٧ جزء في المليون (AI(3) ، والتفاعل بين مختلف معاملات الأسمدة والري السابقة، وقد أظهرت النتائج أن كل القياسات الخضرية (الوزن الطازج والجاف للنبات) قد زادت معنويا بجميع معاملات التسميد الحيوي بالمقارنة بالكنترول، بينما نقصت معنويا بمعاملات الري التبادلي بالمقارنة بـ (AI(1) ، وقد أظهرت التحليلات الإحصائية أن التداخل بين المعاملات المختلفة أدت إلى زيادة محصول العشب الطازج والجاف للنبات، كما أظهرت النتائج ان كل معاملات التداخل أدت إلى زيادة محصول الزيت العطري معنويا، وزيادة كل من الكربوهيدرات والنيتروجين والفوسفور والبوتاسيوم (%) في التجربة، كما أظهرت النتائج أن أفضل المعاملات تأثيرا على المحصول ومحتوى الزيت العطري والكربوهيدرات والنيتروجين والفوسفور والبوتاسيوم هي استخدام معاملة التفاعل بين معاملة الخليط بين بكتريا *Azotobacter* and *Bacillus* (A+B) مع الري مرة واحدة بماء ملوحتة ٣٩٧ جزء في المليون يعقبه الري مرة واحدة بماء ملوحتة ٣١١٧ جزء في المليون (AI(1) تحت ظروف شمال سيناء.

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