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Alleviating Salt Stress in *Thymus capitatus* plant using plant growth-promoting bacteria (PGPR)

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Abstract : This study is to evaluate the effect of Plant-Growth Promoting bacteria (PGPR) (*Azospirillum lipoferum* and/or *Bacillus megaterium*) on *Thymus capitatus* L. grown under saline irrigation conditions (control (fresh water), S1 = 3.13 dSm-1 and S2 = 6.25 dSm-1). The experiments were conducted under normal environmental conditions during winter seasons of 2014 and 2015 at the green house of the National Research Center, Dokki, Cairo, Egypt. The analysis of the data collected during the study indicated that there were statistically significant increases in plant growth, yield, photosynthetic pigments and some chemical contents of thyme plant with different PGPR treatments especially under saline irrigation, which revealed significant decreases in the previously mentioned characters. While reverse trend was obtained for proline content, which increased significantly by increasing salinity levels and revealed significant decreases with all PGPR treatments.
Key words: Salinity, PGPR, growth, yield, total carbohydrates, Proline, photothynthetic pigments, RWC%.

Introduction

Salinization of soils or waters is one of the world's most serious environmental problems in agriculture. It is necessary to determine the environmental factors under which medicinal and aromatic plants give higher yields and better quality. The problem of salinity is caused by poor quality of irrigation water or due to higher rates of evapotranspiration and lack of leaching water(Shao *et al*., [1]). The negative effects of salinity stress on plant-growth include aninhibition in growthrate and biomass, shorter stature, smaller leaves, osmotic effects,nutritional deficiency and mineral disorders **(**Parida and Das, [2] and El-Bassiouny and Abdel-Monem,[3]).In general, salt stress decreases the photosynthesis and respiration rate of plants. Total carbohydrate, fatty acid and protein content were adversely affected due to salinity, but increased the level of amino acids, particularly proline(Jamil *et al*., [4]).Presence of salts in the soil hinders the plant growth mainly by two reasons; First, by osmotic or water-deficit effect of salinity i.e. reduction of water uptake by the plant. Second, by ion-excess effect of salinity excessive accumulation of salts in the plants system causes injury to the plant cells (Avinash, [5]). In addition, Tester and Davenport 6mentioned that salts compete with the various nutrients at various levels of crop growth and development resulting in nutrient deficiency and ion toxicity**.**

Thymus capitatus is a compact, woody perennial native to Mediterranean Europe and Turkey. It is also known under the name *Thymbra capitata*. The plant has with rising stems and narrow, fleshy, oil-gland-dotted, green leaves to 12 mm (0.47 in) long. Thyme species belong to Lamiaceae family (Ismaili *et al.,* [7]). Many of them are used as food preservative (Dababneh, [8]). It also possesses antispasmodic, antiseptic, expectorant, carminative and ant oxidative properties (Dapkevicius *et al*, [9]*).* The main constituents of thyme include thymol, carvacrol and flavonoids which have anti-bacterial, antioxidant, anti-flatulent and anti-worm characteristic **(**Dob

et al.[10]).In many areas, different plant parts were used as powder to treat digestive disorder, diarrhea, fever, coughs and cold (Abdelaziz *et al*,[11]).

Regarding the importance of medicinal plants and their role in human health, it is imperative to increase their biomass without application of harmful chemical fertilizers. Biological fertilizers are organic products containing living cells of different types of microorganisms, which have the ability to convert nutritionally important elements from unavailable to available form through biological processes (Sokhangoy*et al.,* [12]). Different Plant-Growth Promoting Rhizobacteria (PGPR) is known such as Azospirillum, Azotobacter, Bacillus, Clostridium and Pseudomonas has been used for their beneficial effects (Ozturk *et al.*,[13]). Several studies have clearly showed positive effect of PGPR on growth of different crops in different climates and soils (Salantur *et al*., [14] and Mohsen and Ismail, [15]). Effects of Azospirillum on yield of several crops have been reviewed (Bhaskara and Charyulu,[16]).Mode of action of Azospirillum on plants is secretion of phytohormones, fixation of atmospheric nitrogen, reduction of nitrate and the enhancement of mineral uptake (James, [17]).*Azospirillum lipoferum*, were found to have the ability to release phytohormones similar to gibberellic acid and indole acetic acid, which could stimulate plant growth, absorption of nutrients, and photosynthesis (Fayez *et al.*, [18]).The use of rhizospheric microorganisms as Bacillus sp. is the best biological means to enhance solubility of phosphate in the soil and to provide sufficient quantities for plant nutrition(Singh *et al.,*[19]). In saline environments, microorganisms need to balance the osmotic pressure between intra and extracellular. Osmotic adjustment is achieved by increasing solute concentration inside the cell by the accumulation of organic and inorganic solutes. The *Bacillus* species respond to elevated ionic strength media by accumulating any variety of osmolytes including proline, glutamic acid, various ectoines and glycine betaine. Among these organic molecules, amino acids are substrates of choice accumulated to face the stress (Ventosa *et al.*,[20]).

Therefore, the main target of the present work is to improve adaptive features of *Thymus capitatus* that allow it to grow and survive under saline irrigation conditions by using two Plant-Growth Promoting Rhizobacteria (PGPR) *Azospirillum lipoferum* and/or *Bacillus megaterium*.

Materials and Methods

Two pot experiments were conducted during two winter seasons of 2014 and 2015 at the green house of the National Research Center, Dokki, Cairo, Egypt. Seeds of thyme were kindly provided by "SEKEM" company and planted in the nursery on 15th of January. The seedlings of thyme plants were transplanted from the nursery to the permanent soil in March. Uniform rooted plantlets (3 weeks old) were transplanted in earthenware pots 30 cm diameter and 40 cm height with perforated bottoms. All pots were filled with 10 Kg of sandy loam soil, the physical and chemical properties of the soil used were done according to the methods described by Jackson [21].The soil type was sandy loam in texture with water holding capacity 29.0%, pH 7.8, O.M 0.35% and E.C. 1.15 dSm^{-1}.The soil analysis, 0.55% containing $CaCO_3$, available 4.46, 23.46, 169 and 32.2 mg 100 g^{-1} soil of P, K, Mg and Na, respectively and also available 7.2, 9.4, 2.80 and 4.82 ppm of Fe, Mn, Cu and Zn, respectively. One plantlet was planted in each pot in both seasons. Plantlets were irrigated regularly with fresh water for two weeks after transplanting, and then seedlings were subjected to different salinity levels by dissolving sea salts till they reached the EC, S1 = 3.13 dSm^{-1} and S2 = 6.25 dSm^{-1}. The irrigation whether with fresh water or saline water must reach the level of 65% of total Water Holding Capacity (W.H.C.) of the soil by weighing every pot daily and the needed amount of water was added. The general principal stated by Boutraa and Sanders [22] was used for the water treatment application. Three weeks after transplanting, plantlets were inoculated with either of *Azospirillum lipoferum* and/or *Bacillus megaterium* or maintained as un-inoculated controls. Active strain of *Azospirillum lipoferum* and *Bacillus megaterium* provided by the Unit of Bio fertilizers, Faculty of Agriculture, Ain Shams University, Shobra El-Kheima, Egypt. The soil was inoculated with *Azospirillum lipoferum* and/or *Bacillus megaterium*two times (the first three weeks after transplanting, while the second one month later). Cell suspension of *B. megaterium or Azospirillum lipoferum* was grown on nutrient agar medium (Manual, [23]) and applied single or mixed strains according to treatment used. Mixed cultures of bacterial species, containing I × 106 colony forming unitsmL^{-1}, were used for plant inoculation. In addition, the aqueous extract from the raw materials (rice, broad bean, maize, wheat & white clover straws treated with cellulose decomposers) were made and analyzed chemically before application to the soil. For bio fertilizer treatments, each pot was inoculated with 10 mL bacterial suspension of (*Azospirillum lipoferum*) and/or *B megaterium.*

The experiment including 12 treatments which were the combination between three salinity levels (0 "fresh water", S1 = 3.13 dSm^{-1} and S2 = 6.25 dSm^{-1}) and the three inoculations of PGPR plus uninoculated control (B0=control, B1= *Azospirillum lipoferum*, B2=*Bacillus megaterium*, B3= *Azospirillum+Bacillus*). The treatments under investigation were arranged in a complete Randomized Block Design (CRBD) with three replicates. The three salinity levels represented the main plots, while the four PGPR inoculations represented in sub-plots.

The plant herbage was harvested by cutting 5 cm above the soil surface in two separated cuts (in June and November) in both seasons. At each cut, three plants were selected randomly from three separated pots and the following growth parameters were recorded: plant height (cm), number of branches, fresh and dry weights of whole herb (g) and oil% were recorded. The relative water content percent was measured also on fresh leaves according to Barrs and Weatherly [24]. Samples were collected and dried for 48 h at 70 °C to determine the chemical constituents of leaves:

Photosynthetic Pigments: It was determined according to Metzner *et al*. [25].

Proline Content: was determine as (uMole/g dry weight) on dry leaves according to Troll [26].

Total Carbohydrate %: Extraction and determination of total carbohydrates were carried out according to Dubois*et al.* [27].

Quantitative determination of thyme essential oil percentage of the different treatments was achieved by Hydro-distillation according to (Guenther, [28]). Fresh herb of each treatment was subjected to hydro distillation for 3 hours after water boiling till no further increase in the oil was observed. Oil (%) = observed volume of oil (mL)/weight of sample (g) × 100

Statistical analysis was performed according to Snedecor and Cochran [29]. Treatments mean were compared by L.S.D test at 5% level. Combined analysis was made from the two growing seasons hence the results of two seasons followed similar trend.

Results and Discussion

Morphological characteristics and development:

The data presented in Table 1 proved that there was a significant decline in all growth characters of *Thymus capitatus* plant as the concentration of NaCl increased in irrigation water. Plant height, number of branches/plant, fresh and dry weights of the whole plant decreased significantly with increasing salinity level in both cuts of the two growing seasons as compared with control plants. Where, the highest salinity level (S2) recorded lower values of: plant height (50.75 and 47.25 for both cuts respectively), number of branches (21 and 18), fresh weight (100 and 60.50) and dry weight (42.75 and 37.75) than that recorded by control treatment (S0) which were 72 and 56.75 for plant height in both cuts respectively, 31.75 and 26.75 for number of branches, 153.75 and 161.50 for fresh weight and 67 and 66 for dry weight and with significant differences. The main reason for the reduction in herb growth and yield may be attributed to that salinity osmotically induced water stress, specific ion toxicity due to higher concentration of Na^+ and $C1^-$, Nutrient ion imbalance due to high level of Na^+ and $C1^-$ which reduce the uptake of K^+, NO_3^-, PO_4^{2-} *etc* and increased production of reactive oxygen species which damage the macromolecules. Salt stress reduces ability of plants to take up water, and this leads to reduction in growth. Moreover, salinity affects the growth by slow or less mobilization of reserve foods, suspending the cell division, enlarging and injuring hypocotyls (Rahman *et al.*,[30]).Reduced growth has been reported on thyme basil, chamomile and marjoram by (Ramin, [31]; Ali*et al*., [32]; Belaqziz *et al.*,[33] and Baatour *et al*., [34] respectively). The reduction in dry weight may be attributed to inhibition of hydrolysis of reserved foods and their translocation to the growing shoots. Khammari *et al.*, 35 showed that increasing salinity stresses caused reduction in both shoot and root yield of Nigella sativa. Similar decreases in fresh and dry weights under salt stress were found in *Withania somnifera* and *Catharanthus roseus* (Jaleel *et al.*,[36] and Jaleel *et al.*,[37]); *Achillea fragratissima* (Abd EL-Azim and Ahmed, [38]); *Salvia officinalis* (Ben Taarit*et al.*,[39]); thyme (Ezz El-Din *et al.*,[40]); *Nigellasativa* (Hussain *et al.*, [41]); *Chamomilla recutita* (Ghanavati and Sengul, [42]); and basil (Said-Al Ahl and Mahmoud,[43]) and Gholizadeh *et al*, [44] reported similar results on some medicinal plants.

Table (1):Effect of salinity and PGPR treatments on growth and yield of *Thymus capitatus* during the two growing seasons.

Characters / Treatments		Plant height (cm)		No of branches		Fresh weight (g)		Dry weight (g)	
		1st cut	2nd cut	1st cut	2nd cut	1st cut	2nd cut	1st cut	2nd cut
Salinity									
S0		72.00	57.75	31.75	26.75	153.75	161.50	67.00	66.00
S1		58.25	56.75	29.50	22.50	118.75	99.25	52.25	41.00
S2		50.75	47.25	21.00	18.00	100.00	60.50	42.75	37.75
$LSD_{0.05}$		4.26	3.48	2.97	4.01	5.09	6.21	4.62	3.98
PGPR treatments									
B0		46.00	40.33	19.67	18.33	90.33	83.00	44.33	36.00
B1		61.33	52.67	26.67	22.00	109.00	97.00	51.00	42.00
B2		65.33	60.00	30.00	24.00	145.33	123.33	60.33	57.00
B3		68.67	62.67	33.33	25.33	152.00	125.00	60.33	58.00
$LSD_{0.05}$		2.07	4.97	4.36	3.78	4.88	3.81	2.11	3.32
Salinity X PGPR treatments									
S0	B0	59	43	25	23	92	114	53	40
	B1	72	52	30	26	161	135	69	47
	B2	76	65	32	28	176	198	70	88
	B3	81	67	40	30	186	199	76	89
S1	B0	40	40	20	20	95	83	40	33
	B1	60	60	28	22	107	100	43	42
	B2	65	65	34	24	133	106	61	44
	B3	68	66	36	24	140	108	65	45
S2	B0	39	38	14	12	84	52	40	35
	B1	52	46	22	18	59	56	41	37
	B2	55	50	24	20	127	66	44	39
	B3	57	55	24	22	130	68	46	40
$LSD_{0.05}$		5.01	4.69	3.54	4.08	6.78	7.16	6.05	5.21

S0 = irrigation with fresh water, S1 = 3.13 dsm^{-1}, S2=6.25 dsm^{-1}
B0=uninoculated control. B1=.*Azospirillum lipoferum treatment*, B2= *Bacillus megaterium treatment*. B3= *Azospirillum lipoferum + Bacillus megaterium treatment*.

Application of nitrogen fixer strain (*A. Lipoferum)* and phosphate solubilizing bacteria B. *megaterium* alone or in combination induced significant increase in growth parameters of *Thymus capitatus* in terms of plant height (cm), number of branches / plant, as well as fresh and dry weights of herb in the two cutting during the two growing seasons (Table 1). Such promoting effect was maximal by using the combined treatment of *Azospirillum lipoferum* and *Bacillus megaterium* (B3) and gave better plant growth than those obtained from either unioculated control or bio-fertilizer alone during the two cutting of both growing seasons, followed by *Bacillus* treatment (B2). Interaction of specific bacterium to facilitate plant development might be due to either direct or indirect stimulation. Direct one includes biological nitrogen fixation, producing phytohormones like auxins, cytokinins and gibberellins, solubilizing minerals like phosphorus and iron, production of siderophores and enzymes and induction of systemic resistance, while indirect stimulation was basically related to biocontrol, including antibiotic production, chelation of available Fe in the rhizosphere, synthesis of extracellular enzymes to hydrolyze the fungal cell wall and competition for niches within the rhizosphere (Zahir *et al.,*[45] and Gharib *et al*., [46]). Also, *Bacillus megaterium* strains were the most powerful phosphate solubilizes. Furthermore, it was found that microbial inoculation not only increased the nutritional assimilation of the plants, but also improved soil properties, such as organic matter and total N-content (Zahiroddini*et al.,*[47]). The combination between *Azospirillum sp.* and *Bacillus sp.* was the best combination for PGPR-mediated indirect plant growth stimulations (Damayanti *et al.,*[48]). Besides nitrogen transformation, increasing bioavailability of phosphate, iron acquisition, exhibition of specific enzymatic activity and plant protection from harmful pathogens with the production of antibiotics. Similar findings had been obtained by Lucy *et al.,* [49]; Gray and Smith [50]; Banchio *et al,* [51], Hassan *et al.,* [52],Shahzad *et al.*[53] and Abo-Kora and Mohsen [54].

According to the interaction between various salinity levels and different PGPR inoculation the data in Table 1 indicated that all parameters under investigation significantly responded to all applied microorganisms treatments under different salinity levels compared with control plants. While, the highest significant means obtained in S0XB3 treatment, followed by single inoculation with Bacillus (S0XB2) under the same irrigation level as compared with control. The minimum significant means were found under the effect of S2XB0 treatment compared with the other treatments. Numerous soil beneficial bacteria exhibited strong growth adaptation potential under stressful condition. An explanation for the ameliorating phenomenon induced by microorganism's treatments in thyme plant under salinity stress might be due to that the PGPR inoculum enhanced water uptake. The long-term goal of improving plant–microbe interactions for salinity-affected plants and crop productivity could be met with an understanding of the mechanism of osmo adaptation .The synthesis and activity of nitrogenases was inhibited by salinity stress (Tripathi *et al.*,[55]). Tripathi *et al.*[55] documented that in Azospirillum sp. there was an accumulation of glutamate, proline, glycine betaine and trehalose in response to salinity/osmolarity; proline played a major role in osmoadaptation through increase in osmotic stress that shifts the dominant osmolyte from glutamate to proline. Which lead to more water content, higher water potential and lower canopy temperature in their foliage. So, they were less stressed over uninoculated plants. Creus *et al.*[56] illustrated that turgor pressure at low water potential was higher in inoculated two wheat cultivars under osmotic stress. This may be due to better water uptake induced by inoculation that, in turn, was reflected in faster shoot growth in inoculated seedlings exposed to these stresses. Similar increases in growth and yield parameters as a result of PGPR treatments were recorded by Fischer *et al.*[57]; Bacilio *et al.*[58], Bacilio *et al.*[59] and Hashem *et al.*[60].

Photosynthetic pigments:

Table 2:Effect of salinity and PGPR treatments on photosynthetic pigments of *Thymus capitatus* during the two growing seasons.

Characters / Treatments		Chl. a		Chl. b		Chl. a+b		carotenoids	
		1st cut	2nd cut	1st cut	2nd cut	1st cut	2nd cut	1st cut	2nd cut
Salinity									
S0		3.88	3.27	3.86	3.49	7.75	6.76	4.67	5.58
S1		2.45	2.28	2.42	2.25	4.87	4.53	5.68	6.42
S2		1.48	1.27	1.25	1.16	2.73	2.43	6.77	7.19
$LSD_{0.05}$		0.23	0.11	0.31	0.19	0.36	0.41	0.46	0.52
PGPR Treatments									
B0		1.54	1.46	1.58	1.47	3.12	2.93	3.43	3.97
B1		2.25	2.03	2.16	1.94	4.42	3.98	4.40	5.46
B2		2.82	2.64	2.53	2.26	5.35	4.90	5.58	6.52
B3		3.80	2.97	3.77	3.53	7.57	6.50	9.39	9.63
$LSD_{0.05}$		0.46	0.51	0.43	0.35	0.53	0.46	0.55	0.59
Salinity XGPR Treatments									
S0	B0	2.02	2.00	2.02	2.01	4.03	4.01	2.49	2.65
	B1	3.67	3.22	3.56	3.12	7.23	6.34	3.18	4.55
	B2	3.74	3.52	3.79	3.23	7.53	6.75	4.16	5.34
	B3	6.12	4.34	6.08	5.60	12.20	9.93	8.82	9.34
S1	B0	2.11	2.04	1.98	1.78	4.09	3.82	3.12	4.25
	B1	2.26	2.11	2.19	2.01	4.46	4.12	4.62	4.97
	B2	2.65	2.42	2.73	2.54	5.38	4.96	5.36	6.66
	B3	2.77	2.56	2.78	2.67	5.55	5.23	9.60	9.78
S2	B0	0.50	0.34	0.74	0.61	1.23	0.95	4.69	5.00
	B1	0.84	0.77	0.74	0.70	1.57	1.47	5.38	6.88
	B2	2.08	1.98	1.05	1.00	3.14	2.98	7.23	7.55
	B3	2.50	2.00	2.47	2.33	4.97	4.33	9.75	9.78
$LSD_{0.05}$		0.44	0.64	0.25	0.37	0.21	0.46	0.33	0.22

S0 = irrigation with fresh water, S1 = 3.13 dsm^{-1}, S2=6.25 dsm^{-1}

B0=uninoculated control. B1=.*Azospirillum lipoferum treatment*, B2=*Bacillus megaterium treatment*.B3= *Azospirillum lipoferum +Bacillus megaterium treatment*.

It is evident from data in Table 2 that increasing salinity concentration in irrigation water caused significant decreases in Chla, Chlb and total chlorophyll (Chla+b)concentration of *Thymus capitatus* leaves compared with control plants in both cuts of the two growing seasons, where the maximum records were obtained under the control treatment S0. Furthermore, the minimum records obtained under the highest salinity level S2 and with significant differences. While, opposite trend was obtained for the carotenoids content which revealed significant and progressive increase in their content with increase in salinity levels, where the highest significant means were observed under the highest salinity level S2 compared with the other two treatments. The decrease in chlorophyll content under salinity was supported by several authors such as Azooz *et al.*[61] on sorghum, Dager *et al.*[62] on Salvadora, Grewal [63] on persica and Reza *et al.*, [64] on chickpea**.** While, this result may be due to reduction in leaf area, it also can be an adaptive response to reduce the harmful effects of salinity stress (Farooq*et al.*,[65]).Or it may be attributed to both the increase in degradation and the inhibition of pigment synthesis (Garsia-Sanchez *et al.*,[66]).It may also attribute to the disturbance of ions absorption involved in chloroplast formation and protein synthesis and/or plastid breakdown (Abd El-Wahab,[67]). Higher level of carotenoid concentration in stressed plants has also been reported by Deng *et al.*[68]; Kalefetoglu Macer and Ekmekci [69].Stressed plants showed highest carotenoids content than control plants. Carotenoids participate in energy dissipation and could aid plant resistance against stress conditions (Gunes *et al.*,[70]).Increase in carotenoids content for osmotic adjustment in salinity-stressed leaves in many plants has been reported by Khan *et al.*,[71]and Gunes *et al.*,[70]. It had a positive effect on the RWC. High RWC might result from osmoregulation by osmoprotectants, as carotenoids or sugars were accumulated in plants subjected to stress conditions (Leport *et al.*, [72]; Franca *et al.*[73], Gunes *et al.*,[70] and Reza *et al.*,[64]).

The different PGPR treatments had a significant effect on chlorophyll *a*, chlorophyll *b*, chlorophyll *a+b* and carotenoids content of the thyme leaves as shown in Table 2.The obtained values of the photosynthetic pigments showed that use of different types of beneficial microorganisms alone or with a mixture caused significant increases in all photosynthetic pigments content compared with uninoculated plants in both cuts and during the two growing seasons. The more pronounced effect was obtained under the effect of mixed inocula of *Bacillus megaterium* and *Azospirillum lipoferum* (B3) compared with single inoculation and uninoculated plants, followed by single inoculation with *Bacillus megaterium.* These results were in greeting accordance with those obtained by Badran and Safwat [74] on fennel plants, Ali[75] on fennel, Abo El-Fetooh and Hanaa[76] on *Calendula officinalis* plantsand Abo-Kora and Mohsen[54] on sweet basil plant. These effects assigned to microbial activities in synthesis of phytohormones, organic acids and vitamins, nitrogen fixing, increased some nutrients availability like phosphorus and finally interactions between PGPRs and other soil microorganisms in the rhizosphere which benefits the plant growth (Barea *et al.*,[77]and Chen,[78]).

Moreover, different microorganism's inoculation showed marked increases in concentrations of chlorophyll a chlorophyll *b*, chlorophyll *a+b* and carotenoids content of the thyme leaves under different salinity levels compared with control plants. The highest mean values of chlorophyll a chlorophyll *b* and chlorophyll *a+b* of the two cuts were found under the effect of S0XB3 treatment and with significant difference compared with their controls and the other interactions. While, the highest significant values of carotenoids content were observed in S3XB3 treatments compared with the other treatments. The ameliorative effects of beneficial microorganisms on plant under saline irrigation conditions have been listed by Zahir *et al.*[79]; Egamberdieva[80] and Rojas-Tapias *et al.*[81].

Relative water content (RWC) %:

Regarding the effect of different salinity levels on thyme leaf relative water content%, the obtained data in Table 3 revealed that there was a gradual significant decrease in RWC% as salt concentration in irrigation water increased. High salinity levels S2 showed the highest significant reduction in RWC% compared to the control plants in both cuts during the two growing seasons.

The percentages of reduction in RWC% were 30 and33.32% for both cuts respectively compared to control. Similar results have been reported by other scientists; Soliman *et al.*[82] on five Apiaceae species; Salwa*et al.*[83] on peanut plant and Ahmad *et al.*,[84] on mustard cultivars. In saline soils, uptake of water by plants is driven by the difference in water potential between the soil and plant roots. Decreased soil water potential under high salinity restricts water flow into plants roots, which reduces pressure-driven xylem transport of water to aboveground tissues due to altered membrane permeability, which in turn caused decrease in RWC%. Stress leads to increase accumulation of reactive oxygen species such as superoxide, hydrogen peroxide and the

hydroxyl radical. They caused damage to cell membrane structure by injuring cell components that ultimately electrolyte leakage was increased (Foyer, [85]). Khodary[86] concluded that water stress caused to change in phospholipid membranes and increased unsaturated acids and therefore, increased electrolyte leakage. Increased salt content caused negatively effect on plant water relations and water content, and RWC decreasing as salt increases in irrigation water (Lee *et al.*,[87]).

Table (3):Effect of salinity and PGPR treatments on RWC%, Oil%, Carbohydrates %and Proline content of *Thymus capitatus* during the two growing seasons.

Characters / Treatments		RWC%		Oil%		Carbohydrates%		Proline uMole/g dry weight	
		1st cut	2nd cut	1st cut	2nd cut	1st cut	2nd cut	1st cut	2nd cut
Salinity levels									
S0		72.42	67.64	1.63	1.43	70.55	70.44	0.083	0.082
S1		55.85	54.94	1.89	1.70	64.16	63.86	0.093	0.105
S2		50.60	45.10	2.50	2.18	57.62	58.02	0.105	0.121
$LSD_{0.05}$		4.21	6.12	0.47	0.22	4.21	3.41	0.022	0.011
PGPR Treatments									
B0		53.46	50.77	1.52	1.42	61.37	60.72	0.114	0.136
B1		57.51	52.18	1.99	1.69	63.22	62.58	0.097	0.101
B2		60.62	55.99	2.08	1.94	64.62	65.78	0.079	0.076
B3		66.90	64.63	2.43	2.04	67.21	67.34	0.085	0.097
$LSD_{0.05}$		3.22	4.26	0.07	0.10	2.64	3.22	0.008	0.014
Salinity X PGPR Treatments									
S0	B0	65.24	60.53	1.08	1.00	68.20	66.46	0.107	0.116
	B1	70.56	61.93	1.66	1.42	69.78	67.15	0.091	0.081
	B2	74.87	68.45	1.79	1.62	71.08	73.21	0.058	0.043
	B3	79.00	79.65	2.00	1.67	73.12	74.95	0.077	0.085
S1	B0	50.23	50.23	1.44	1.43	62.43	60.25	0.111	0.135
	B1	51.75	50.33	1.87	1.63	63.41	63.17	0.099	0.100
	B2	54.33	53.23	1.89	1.83	64.32	65.13	0.085	0.090
	B3	67.11	65.99	2.35	1.89	66.48	66.87	0.079	0.095
S2	B0	44.92	41.57	2.04	1.83	53.48	55.46	0.124	0.155
	B1	50.21	44.29	2.45	2.00	56.48	57.41	0.101	0.123
	B2	52.67	46.30	2.56	2.36	58.46	58.99	0.095	0.095
	B3	54.60	48.24	2.95	2.57	62.04	60.21	0.100	0.110
$LSD_{0.05}$		5.02	4.01	0.09	0.08	4.21	2.11	0.009	0.011

S0 = irrigation with fresh water, S1 = 3.13 dsm-1, S2=6.25 dsm-1
B0=uninoculated control. B1=.*Azospirillum lipoferum treatment*, B2=*Bacillus megaterium treatment*.B3= *Azospirillum lipoferum +Bacillus megaterium treatment*.

Results of this study also showed that *Azospirillum* and/or *B. megaterium* increased RWC% of thyme leaves significantly as compared to control (Table 3). Where, the non-inoculated control plants were significantly deficient in their relative water content as compared to the *Azospirillum lipoferum* and *B. megaterium* treatments alone or in combination. Moreover, the combination of *Azospirillum lipoferum* with *B. megaterium* showed higher RWC% means compared to the control plants and with significant differences. Our findings were in confirmatory to other studies recorded by Zhang *et al.*[88], Garg & Manchanda, [89] and Rakshapal *et al.*[90]. The observed increase in RWC% might be due to the integrity and stability of cellular tissues by the PGPR interactions compared with non-PGPR interactions (Garg & Manchanda, [89]). Although, other studies mentioned that PGPR inoculation stimulate ABA accumulation in leaves and roots of stressed plants as compared to non-inoculation control (Herrera-Medina *et al.*, [91]). Protective effect of ABA was pivotal in RWC% as it promoted stomata closure to reduce water loss and mediates stress damage through activation of many stress-responsive genes, which collectively increases the plant's tolerance (Zhang *et al.*,[88]).

As for the effect of dual-interaction, the data illustrated that inoculated plants with *Azospirillum lipoferum* and/or *B. megaterium* revealed significant increases in RWC% values under different salinity levels compared with their control and with significant differences in both cuts. The data in Table 3 revealed also that the highest RWC% values observed under S0XB3interaction and with significant differences compared with

their control and other interactions of both cuts during the two growing seasons. While, the lowest means observed in uninoculated plants under the highest salinity level S2 i.e S2XB0 treatment. Our results were in line to other studies of Rakshapal *et al.*[90] and Garg & Manchanda,[89] who revealed that the PGPR-inoculated plants not only reduce stress effect but also help to fetch higher water quantity compared with control plants. Thus, indicating that the beneficial association could help plant to tolerate such stresses.

Essential oil percentage:

Examination of the collected data in Table 3 revealed significant increases in essential oil percentage due to increase in salinity level compared with control plants in both cuts and in both growing seasons. Where, S2 treatment revealed the highest significant increase in oil% content of thyme leaves, the values reached (2.5 and 2.18 for 1st and 2nd cuts respectively) compared with the other treatments. Where, the percent of increases reached to 53.37 and 52% for the 1st and 2nd cuts respectively compared with control treatments. While, the lowest means were obtained under the control treatment S0 (which were 1.63 and1.43for both cuts respectively).Similar results of a stimulatory effect of high salinity level were also found on *Satureja hortensis* (Baher *et al*., [92]); sage (Hendawy and Khalid, [93]) and thyme (Ezz El-Din *etal.*,[40]). Furthermore, Said-Al Ahl and Mahmoud[43] recorded on basil that the highest oil percentage was achieved under salinity conditions. The stimulation of essential oil production under salinity could be due to a higher oil gland density and an increase in the absolute number of glands produced prior to leaf emergence (Charles *et al.*, [94]).

It is interesting also to note that, there were significant effects of all PGPR treatments on oil% compared with untreated plants in both cuts. Inoculating the thyme plants with *Azospirillum lipoferum* and/or *B. megaterium* resulted in a higher oil% in comparison to control. However, the highest significant increase in oil% appeared under the combined treatment of *Azospirillum lipoferum* and *B. megaterium* (B3) compared with the other treatments and the control, followed by *B. megaterium* (B2) treatment. Similar trend was obtained for the second season. These results were somewhat similar to those obtained by Edris *et al*. [95]who found that the relative percentage of certain constituents of marjoram essential oil was affected by bio fertilization type and level. Also, Scavroni *et al*. [96] reported increase in oil yield but did not improve oil quality of *Mentha piperita.* Moreover, Hassan *et al.*[52] recorded increase in growth characters and essential oil composition of coriander plants treated with bio fertilizers compared with that treated with the chemical fertilizers. The most notable phenomenon in *Azospirillum* inoculation that it worked better when phosphate-solubilizing bacteria such Azotobacter, rhizobia, bacilli, and VAM fungi were incorporated, perhaps helping the growth of each other by synergistically providing nutrients, removing inhibitory products, stimulating plants' ability to grow better, apparently co-inoculation allows plants to achieve a more balanced nutrition and (or) absorption of nutrients was improved. It enhanced quality characteristics of oil and yield, higher net return, and better cost-benefit ratio (Bashan *et al.*,[97] and Abo-Kora and Mohsen,[54]).

For the effect of interaction between the different salinity levels and different PGPR treatments the obtained data showed that the different PGPR treatments caused significant increases in oil% means under different salinity levels, where the highest significant oil% content appeared under the combined effect of S2XB3 treatment, followed by S2XB2 treatment compared with the other interactions, these results were true in both cuts and in the both growing seasons. Our results were fortified by those of Plazinski and Rolf [98]; Remans *et al*., [99]; Egamberdieva and Kucharova[100]. Furthermore, Bianco and Defez [101] reported that IAA produced by these microorganisms enhancing different cellular defense systems for protection plants from external adverse conditions. Other researchers have shown that *Serratia* sp. and *Rhizobium* sp. ameliorated the deleterious effect of salinity on growth, yield and enzymes activities of lettuce (Han and Lee, [102]). The levels of phytohormones play an important role in protection of the plant against various stresses. The low concentration of pure IAA or low titer of IAA producing bacteria enhanced plant growth and yield. Khalid *et al*.[103] also reported that plants treated with PGPR showed an increase in the main components of the essential oil (limonene and β-selinene)of Celery plant comparison to untreated plants.

Total carbohydrates %:

It could be seen that the contents of carbohydrates in the leaves of thyme plant tends to decrease with increasing salinity level (Table 3). Where, the highest significant records in their contents obtained under control treatment S0 compared to the other treatments in both cuts of the two growing seasons. While, the lowest means obtained under S2 treatment. The percent of reduction in carbohydrates content reached to 18.33

and 17.63 % for 1st and 2nd cuts respectively as compared to control. Different studies showed similar results on *Sorghum bicolor* L. (Faheed, [04]), *portulaca oleracea* L. (Yazici, [105]) *Oriza sativa* L. (Amirjani,[106]). The reduction in carbohydrates content as response to salinity could be attributed to the nutritional imbalance (Liu and Zhu, [107]) or the specific toxic effects of salinity (Nou*et al.,*[108]),hyperosmotic stress (Greenway and Munns, [109]) and reduced photosynthesis process which may be due to the shortage of the available water which was reflecting in lowering the plant content of soluble carbohydrates (Pasternak, [110]). Also, salinity reduced the thickness of conductive canals (mainly phloem), so that a reduction in the translocation of assimilates toward the developing organs may be occurred (Khalil, [111]). Furthermore, Banon *et al.*,[112] attributed the above decline in total carbohydrates to soil water deficiency which triggers certain chemical stimulus (mostly ABA) through xylem vessels to leaves of stressed plants which caused stomata closure, reduction of each of stomata conductance, CO_2 concentration in leaf tissues, electron transport system, CO_2 fixation, rate of photosynthesis and eventually quantity of photosynthates. These conditions, in the meantime, enhances some plants to increase their respiration rates as a prerequisite to produce both ATP to activate stressed cells, and osmotic soluble substances which reduces cell osmotic potential thus increasing cell water uptake.

Total carbohydrates in the dry plant material were influenced significantly by the bio fertilizer treatments. The use of different types of beneficial bacteria (PGPR) proved significant increase in total carbohydrates %. Where, the highest values of total carbohydrates were found in dual inoculation with *Azospirillum lipoferum* and *B. megaterium* compared with control treatment and with those obtained from either nitrogen fixers (*Azos.*) or phosphate solubilizers B. *megaterium* alone. *B. megaterium* colonization showed generally more pronounced effects on total carbohydrates % than *Azospirillum lipoferum* which revealed the lowest significant means compared with the other PGPR treatments. Several workers found that PGPR treatments increased total carbohydrates% e.g. Khalid *et al.*[103] who stated that chemical composition analysis of treated plants showed an increase in the essential and fixed oil content, total carbohydrates, crude protein and nutrients content (NPKS) and its uptake. Also, Marulanda-Aguirre *et al.*[113],Hashemabad *et al.*[114],Sang-Mo Kang *et al*. [115] and Abo-Kora and Mohsen, [54] reported similar results. The increased levels of total carbohydrates under PGPR treatments was perhaps due to the necessity of its protective role on chloroplast integrity (Tyler *et al.*,[116]) leading to enhanced photosynthesis under salinity, or may due to increase in leaf resistance which revealed improved in leaf conductance under inoculation which may allow better gas exchange and enhancement of photosynthesis(Talaei *et al.*, [117]).

Concerning the effect of dual-interaction between the two studied factors for both growing seasons, the data revealed that all PGPR treatments revealed significant increases in carbohydrates % under different irrigation treatments compared with their controls. The highest significant records for carbohydrates % was obtained under the combined effect of *B. megaterium+ Azospirillum lipoferum* inoculation and fresh water irrigation (S0XB3) compared to the other treatments in both cuts. Followed by single inoculation with *B. megaterium* under the same irrigation level and the different between the two treatments were insignificant. While the lowest means observed in S2XB0 treatment compared with the other treatments. The increase in total carbohydrates % as a result of PGPR application in our search may be due to the increases in chlorophyll a and b or may be due to that PGPR application had enhancing role in cell division, cell elongation producing more leaf area. A similar trend has also been observed in other researchers (Swedrzynska and Sawicka,[118]; Nadeem *et al.*,[119]; Prathibha and Siddalingeshwara, [120]).

Proline content:

The obtained results in Table 3 pointed out that the proline content increased with increasing salinity levels, the percentage of increase ranged between 26.51 and 47.56% compared with control treatment for both cuts respectively. The highest values for proline content was observed under the highest salinity level S2 (0.105 and 0.121for both cuts respectively) compared with control plants and with the other salinity level in both cuts of the two growing seasons. The observed accumulation of proline in the plants grown under saline conditions may be attributed to the enhancement of hydrolysis effect of salinity on protein which led to accumulation of the intermediary substances containing nitrogen such as ammonia, amino acids, amides and urea (Khalil, [111]); she added also that the accumulation of non-toxic substances under saline conditions such as proline, organic acids and pigments may had protective properties. The increases in proline values under stress conditions was to build the defense mechanism which stressed plants take so as to reduce cell osmotic potential, thus increasing cell water uptake with concomitant increases in both cell turgidity and its activity (Abdalla and El-Khoshiban, [121]). The proline accumulated in leaves as a response to salt stress was reported on *Salvia officinalis*,

Trachyspermum ammi, spearmint, chamomile, sweet marjoram, *Catharanthus roseus, Achillea fragratissima*, *Matricaria chamomilla*, sweet fennel, and *Satureja hortensis* (Ali *et al*.,[32]; Hendawy and Khalid, [93]; Al-Amierand Craker, [122]; Zaki *et al*.,[123];Abd EL-Azim and Ahmed [38];Najafi *et al*.,[124];Ashraf, [125]; Osman *et al*. [126] and Cik *et al*.,[127]). The increase in proline content could be attributed also to a decrease in proline oxidase activity under saline conditions (Muthukumarasamy *et al*. 2000 and El-Bassiouny and Abdel-Monem, [3]).

Application of different PGPR treatments caused significant decrease in proline content of *Thymus capitatus* leaves as compared with control plant in both cuts, where the lowest means observed in *Bacillus megaterium* (B2) treatment. Followed by inoculation with *B. megaterium*+ *Azospirillum lipoferum* treatment(B3) compared with the other treatments. Previous results were supported by Levitt [129], Hasegawa *et al.*, [130], Hathout [131], Bashan *et al*., [97] and Hafsa *et al.*, [132] who reported that bio fertilization resulted in decreasing proline content of different plants under stress conditions. This could be explained as PGPR bacteria in the presence of salt could accumulate osmolytes to achieve osmotic adjustment and ensure the stabilization of certain proteins active at the expense of other enzyme activities loss, due to their altered electrostatic properties. Furthermore, Hafsa*et al.* [132] indicated that *Bacillus* species respond to elevated ionic strength media by synthesizing or accumulating any variety of osmolytes including proline, glutamic acid, various cations and glycine betaine. Among these organic molecules, amino acids were substrates of choice accumulated to face the stress. They added that proline and glycine-betaine were the most commonly used solutes in osmoregulation process. Moreover, Tripathi *et al*.[55] documented that Azospirillum sp. had the ability to accumulate compatible solutes such as glutamate, proline, glycine betaine and trehalose in response to salinity/osmolarity; they added that proline plays amajor role in osmic adaptation through increase in osmotic stress that shifts the dominant osmolyte from glutamate to proline in plant. Which lead to more water content, higher water potential and lower canopy temperature in their foliage. Hence, they were less drought-stressed over uninoculated plants. In addition, Bashan *et al*., [97] illustrated that *A. lipoferum* has a salinity-induced glycine betaine transport system which acts as osmo-protection.

In addition, Pots treated with PGPR bacteria showed marked significant decrease in proline content of thyme leaves under different salinity levels compared with their controls in both cuts, the non-inoculated control plants had significantly higher concentration of leaf proline as compared to PGPR treatments. The maximum records observed in uninoculated plants under the highest salinity level (S2XB0) in both growing seasons. While, the minimum records obtained in control treatment (without salinity) as a response to *B. megaterium* inoculation (S0XB2). There have been several studies, which narrate the same findings of PGPR inoculation under stress conditions e.g. Naz *et al.*,[133];Bano and Fatima, [134];Yao *et al.*, [135]; and Chen *et al.*,[136] who correlated proline accumulation with drought and salt tolerance in plants. Introduction of proBA genes derived from *B. subtilis* resulted in production of higher levels of free proline resulting in increased tolerance to osmotic stress in the transgenic plants. Increased production of proline along with decreased electrolyte leakage, maintenance of relative water content of leaves and selective uptake of K ions resulted in salt tolerance in *Zea mays* co inoculated with PGPR. Likewise, Upadhyay *et al.*[137] studied the impact of PGPR inoculation on growth and antioxidant status of wheat under saline conditions and reported that co-inoculation with *B. subtilis* and *Arthrobacter sp.* could alleviate the adverse effects of soil salinity on wheat growth with an increase in dry biomass, total soluble sugars and proline content.

References

1. Shao, HB, Chu LY Jaleel CA., 2008. Water-deficit stress-induced anatomical changes in higher plants. CR Biologies, 331(3):215-25.
2. Parida, SK, Das, AB, 2005. Salt tolerance and salinity effects on plants. Ecotoxicol.Environ. Saf., 60:324–349.
3. El-Bassiouny, HMS and Abdel-Monem AA, 2016.Role of Tryptophan or Prozac (5hydroxytryptamine) on some Osmolytes and Antioxidant defense system of Sunflower cultivars grown in Saline soil. International Journal of ChemTech Research,9(06):107-120.
4. Jamil M, Lee DB, Jung KY, Lee SC, Rha ES,2006. Effect of salt (NaCl) stress on germination and early seedlinggrowth of four vegetables species. JCEA, 7(2):273-282.
5. Avinash, DP, 2013.Alleviating salt stress in crop plants through salt tolerant microbes. International Journal of Science and Research (IJSR), 4(1):1297-1303.

6. Tester, M and Davenport R, 2003. Na+ tolerance and Na+ transport in higher plants. Annals Bot., 91: 503-527.
7. Ismaili, H, Milella L, Frih-Tetouani S, Ilidrissi A, Camporese A, Sosa S, Altinier G, Della,Loggia R, Aquino R, 2004. In vitro topical anti-inflammatory and in vitro antioxidant activities of two extracts of Thymus satureioides leaves. J. Ethnopharmacol., 91: 31-36.
8. Dababneh, BF, 2007. Antimicrobial activity and genetic diversity of thymus species onpathogenic microorganisms. J. Food Agric. Environ., 5(3&4): 158-162.
9. Dapkevicius, A, Van Beek VTA, Lelyveld GP,Van Veldhuizen A, DeGroot A., Linssen JPH, and Venskutonis R, 2002. Isolation and structural elucidation of radical scavengers from Thymus vulgaris leaves. J. Nat. Prod., 65(6): 892-896.
10. Dob, T, Dahmane D, Benabdelkader T, Chelghoum C, 2006. Studies on the essential oil composition and antimicrobial activity of Thymusalgeriensis Boiss et Reut. Int. J. Aromather., 16: 95-100.
11. Abdelaziz,A, Belaqziz R, Bekkouche K and Markouk M,2011. Influence of temperature and water potential on laboratory germination of two Moroccan endemic thymes: Thymus maroccanus Ball. and Thymusbroussonetii Boiss. African Journal of Agricultural Research,6(20): 4740-4745.
12. Sokhangoy, SH, Ansari KH and Eradatmand AD, 2012. Effect of bio-fertilizers on performance of dill (Anethum graveolens). Iranian Journal of Plant Physiology, 2 (4):547-552.
13. Ozturk, A, Caglar O, Sahin F, 2003. Yield response of wheat and barley to inoculation of plant growth promoting rhizobacteria at various levels of nitrogen fertilization. J. Plant Nutr. Soil Sci. 166: 262–266.
14. Salantur, A, Ozturk R, Akten S, 2006. Growth and yield response of spring wheat (Triticum aestivum L.) to inoculation with rhizobacteria. Plant Soil Environ., 52:111–118.
15. Mohsen MA and Ismail H, 2016. Response of Calendula officinalis L. which grown in saline soil to Plant Growth Promoters and Some Organic Substances. International Journal of Pharm Tech Research, 9(4):153-172.
16. Bhaskara, KVand Charyulu PB, 2005. Evaluation of effect of inoculation of Azospirillum on Setaria italica (L.). Afr. J. Biotechnol., 4:989–995.
17. James, EK, 2000. Nitrogen fixation in endophytic and associative symbiosis. Field Crops Res., 65: 197–209.
18. Fayez, M, Emam NF, and Makboul HE, 1985. The possible use of nitrogen fixing Azospirilum as bio fertilizer for wheat plants. Egypt. J. Microbiol., 20(2): 199-206.
19. Singh, SM, Yadav LS, Singh SK, Singh P, Singh PN, Ravindra R, 2011. Phosphate solubilizing ability of two Arctic Aspergillus niger strains. Polar Research, 30, 7283. DOI: 10.3402/polar.v30i0.7283.
20. Ventosa, A, Nieto JJ, Oren A, 1998. Biology of moderately halophilic aerobic bacteria. Microbiol. Mol. Biol. Rev., 62(2): 504-544.
21. Jackson, ML, 1973. Soil Chemical Analysis. Prentice-Hall, Inc. Englewood Cliffs, N.J. New Delhi,India.
22. Boutraa, A and Sande FE, 2001. Influence of water stress on grain yield and vegetative growth of two cultivars of bean (Phaseolus vulgaris L.). Journal of Agronomy and Crop Science, 187, 251.
23. Manual, D, 1985. Dehydrated Culture Media and Reagents for Microbiology, 10th edition, 487-623. Difco Laboratories DefroitMichigan 48232, USA.
24. Barrs, H D and Weatherley PE, 1962. A re-examination of the relative turgidity techniques for estimating water deficits in leaves. Aus. J. Biol. Sci.15: 413-428.
25. Metzner, H, Rau H and Senger H, 1965.Untersuchungen Zur Synchronisier-Barkeit einzelner pigment mangel-Mutantenvon Chlorella. Planta, 65: 186.
26. Troll, WJL,1995. Aphotometric method for defermination of proline. Journal of Biological chemistry, 215, 655-660.
27. Dubois, M, Gilles KA, Hamilton J, Rebers R, and Smith F, 1956. Colorimetric method fordetermination of sugars and related substances. Anal. Chem., 28(3): 350-356.
28. Guenther, E, 1961. The Essential Oils. Van Nostr and Comp. Inc. New York, Vol. III, pp.448.
29. Snedecor, GW and WG Cochran, 1980.Statistical Methods (7th Edn). Iowa state University, Iowa,USA.
30. Rahman, M, Soomro U, Zahoor-Ul-Hag M, Gul Sh.2008. Effects of NaCl salinity on wheat (Triticum aestivum L.) cultivars. World J Agri Sci.,4(3):398-403.
31. Ramin, AA, 2005. Effects of salinity and temperature on germination and seedling establishment of sweet basil (Ocimum basilicum L.). J Herbs Spices Med Plants; 11(4):81-90.
32. Ali, RM, Abbas HM, Kamal RK, 2007. The effects of treatment with polyamines on dry matter, oil and flavonoid contents in salinity stressed chamomile and sweet marjoram. Plant Soil Environ, 53:529-43.

33. Belaqziz, R, Romane A, Abbad A, 2009. Salt stress effects on germination, growth and essential oil content of an endemic thyme species in Morocco (Thymus maroccanus Ball.). J Applied Sci Res, 5(7):858-63.
34. Baatour, OR, Kaddour W, Wannes A, Lachaal M, Marzouk, B 2010.Salt effects on the growth, mineral nutrition, essential oil yield and composition of marjoram (Origanum majorana). Acta Physiol Plant, 32:45-51.
35. Khammari, I, Saran SA and Dahmardeh M, 2007. The effect of salinity on seed germination and growth in six medicinal plants. Iranian J. Med. Aromatic Plants, 23: 331-339.
36. Jaleel, CA, Lakshmanan GMA, Gomathinayagam M, Panneerselvam R, 2008. Triadimef on induced salt stress tolerance in Withania somnifera and its relationship to antioxidant defense system. South African J Bot, 74:126-32.
37. Jaleel, CA, Sankar B, Sridharan R Panneerselvam R, 2008. Soil salinity alters growth, chlorophyll content, and secondary metabolite accumulation in Catharanthus roseus. Turk J Biol., 32:79-83.
38. Abd EL-Azim, WM, Ahmed ST.2009. Effect of salinity and cutting date on growth and chemical constituents of Achillea fragratissima Forssk, under Ras Sudr conditions. Res J Agr Biol Sci., 5(6):1121-9.
39. Ben Taarit, MK, Msaada K, Hosni M, Hammami M, Kchouk E, Marzouk B, 2009.Plant growth, essential oil yield and composition of sage (Salvia officinalis L.) fruits cultivated under salt stress conditions. Ind Crops Prod., 30(3):333-7.
40. Ezz El-Din, AA, Aziz EE, Hendawy SF, Omer EA, 2009. Response of Thymus vulgaris L. to salt stress and alar (B9) in newly reclaimed soil. J Appl Sci Res, 5(12):2165-70.
41. Hussain, K, Majeed A, Nawaz KH, Khizar HB, Nisar MF.2009. Effect of different levels of salinity on growth and ion contents of black seeds (Nigella sativa L.). Curr Res J Biol Sci., 1(3):135-8.
42. Ghanavati, M, Sengul S, 2010. Salinity effect on the germination and some chemical components of Chamomilla recutita L. Asian J Chem., 22(2):859-66.
43. Said-Al Ahl HAH and Mahmoud AA, 2010. Effect of zinc and/or iron foliar application on growth and essential oil of sweet basil (Ocimum basilicum L.) under salt stress. Ozean J Appl Sci., 3(1):97-111.
44. Gholizadeh, F, Manzari-Tavakkoli A, Pazoki A, 2016. Evaluation of salt tolerance on germination stage and morphological characteristics of some medicinal plants artichoke, flax, safflower and coneflower. International Journal of Farming and Allied Sciences, 5(3):229-237.
45. Zahir, ZA, Muhammad A, Frankenberger WTJ, 2004. Plant growth promoting rhizobacteria:applications and perspectives in agriculture. Adv Agron., 81:97–168.
46. Gharib, FA, Moussa LA, Massoud ON, 2008. Effect of compost and biofertilizers on growth, yield and essential oil of sweet marjoram (Majorana hortensis) plant. Int J Agric Biol., 10 (4): 381-387.
47. Zahiroddini, H, Baah J, Absalom W, McAllister TA, 2004. Effect of an inoculant and hydrolytic enzymes on fermentation and nutritive value of whole crop barley silage. Anim. Feed Sci. Technol., 117: 317–330.
48. Damayanti, TA, Pardede H, Mubarik NR, 2007. Utilization of root-colonizing bacteria to protect hot-pepper against Tobacco MosaicTobamovirus. Hayati J Biosci., 14:105–109.
49. Lucy, M, Reed E, Glick BR. 2004. Applications of free living plant growth-promoting rhizobacteria. Antonie Leeuwenhoek, 86: 1-25.
50. Gray, EJ and Smith DL, 2005. Intracellular and extracellular PGPR: commonalities and distinctions in the plant-bacterium signaling processes. Soil Biol Biochem., 37: 395-412.
51. Banchio, E, Bogino PC, Zygadlo J, Giordano W, 2008. Plant growth promoting rhizobacteria improves growth and essential oil yield in Origanum majorana L. Bioch Syst Ecol., 36: 766-771.
52. Hassan, FAS, Ali EF, Mahfouz SA, 2012. Comparison between different fertilization sources, irrigation frequency and their combinations on the growth and yield of coriander plant. Aust J Basic App Sci., 6 (3):600-615.
53. Shahzad, SM, Khalid A, Arif MS, Riaz M, Ashraf M, Iqbal Z, Yasmeen T, 2014. Co-inoculation integrated with P-enriched compost improved nodulation and growth of Chickpea (Cicer arietinum L.) under irrigated and rain fed farming systems. Biol Fertil Soils, 50:1–12.
54. Abo-Kora, HA and Mohsen MA, 2016.Reducing effect of soil salinity through using some strains of Nitrogen fixers bacteria and compost on sweet basil plant., International Journal of PharmTech Research,9(4):187-214.
55. Tripathi, AK, Nagarajan T, Verma SC, Le Rudulier D, 2002. Inhibition of biosynthesis and activity of nitrogenase in Azospirillum brasilense Sp7 under salinity stress. Curr Microbiol, 44:363–367.

56. Creus, CM, Sueldo RJ and Barassi CA, 2004. Water relations and yield in Azospirillum inoculated wheat exposed to drought in the field. Can J. Bot., 82:273–281.ations and perspectives in agriculture. Adv Agron, 81:97–168.
57. Fischer, S, Rivarola V, Mori G, 2000. Colonization of wheat by Azospirillum brasilense Cd is impaired by saline stress. Plant Soil, 225:187–191.
58. Bacilio, M, Vazquez P, Bashan Y, 2003. Alleviation of noxious effects of cattle ranch composts on wheat seed germination by inoculation with Azospirillum spp. Biol Fertil Soils, 38:261–266.
59. Bacilio, M . Hilda R Manuel M, Juan-Pablo H, Yoav B, 2004.Mitigation of salt stress in wheat seedlings by a gfp-tagged Azospirillum lipoferum. Biol Fertil Soils,40: 188–193.
60. Hashem, A. Abd Allah EF, Al-Huqail A, Alshlawi SR, Wirth S and Egamberdieva D, 2015. Impact of plant growth promoting Bacillus subtilis on growth and physiological parameters of Bassia indica grown under salt stress. Pak. J. Bot., 47(5): 1735-1741.
61. Azooz, MM, Shadded MA, Abdel-Latef AA, 2004. The accumulation and compartmentation of proline in relation to salt tolerance of three sorghum cultivars. Indian J. Plant Physiol., 9: 1-8.
62. Dagar, JC, Bhagwan H, Kumar Y,2004. Effect on growth performance and biochemical contents of Salvadora persica when irrigated with water of different salinity. Indian J Plant Physiol, 9: 234-238.
63. Grewal, HS, 2010. Water uptake, water use efficiency, plant growth and ionic balance of wheat, barely, canola and chickpea plants on a sodic vertosol with variable subsoil NaCl salinity. Agricultural Water Management,97: 148-156.
64. Reza, T, Mohammad HE, Nima B, Ezzat K, Khosro M, 2013.Physiological responses of chickpea (Cicer arietinum) genotypes to drought stress.Environmental and Experimental Biology, 11: 9–15
65. Farooq, M, Wahid A, Kobayashi N, Fujita D, Barsa SMA, 2009. Plant drought stress: effects, mechanisms and management.Agron. Sustain. Dev., 29: 153–188.
66. Garsia-Sanchez F, Jufon JL, Carvaial M, Syverstem JP,2002. Gas exchange, chlorophyll and nutrient contents in relation to Na+ and Cl- accumulation in 'Sunburst' mandarin grafted on different rootstocks. Plant Sci., 162:705-712.
67. Abd El-Wahab MA, 2006. The efficiency of using saline and fresh water irrigation as alternating methods of irrigation on the productivity of Foeniculum vulgare Mill subsp. vulgare var. vulgare under North Sinai conditions. Res J Agr Biol Sci., 2(6):571-7.
68. Deng X, Hu ZA, Wang HX, Wen XG, Kuang TY, 2003. A comparison of photosynthetic apparatus of the detached leaves of the resurrection plant Boea hygrometrica with its non-tolerant relative Chirita heterotrichia in response to dehydration and rehydration. Plant. Sci.,165: 851–861.
69. Kalefetoglu Macar, T and Ekmekci Y, 2009. Alterations in photochemical and physiological activities of chickpea (Cicer arietinum L.) cultivars under drought stress. J. Agron. Crop. Sci.,195: 335–346.
70. Gunes A, Inal A, Adak MS, Bagci EG, Cicek N, Eraslan F, 2008. Effect of drought stress implemented at pre- or post- anthesis stage some physiological as screening criteria in chickpea cultivars. Rus. J. Plant. Physiol., 55: 59–67.
71. Khan, AJ, Hassan S, Tariq M, Khan T, 2001. Haploidy breeding and mutagenesis for drought tolerance in wheat. Euphytica,120: 409–414.
72. Leport, L, Turner NC, French RJ, Barr MD, Duda R, Davies SL, Tennant D, Siddique KHM, 1999. Physiological responses of chickpea cultivars to terminal drought in a Mediterranean-type environment. Eur. J. Agron., 11: 279–291.
73. Franca, MGC, Thi ATP, Pimental C, Rossiello ROP, Fodil YZ, Laffray D, 2000. Differences in growth and water relations among Phaseolus vulgaris cultivars in response to induced drought stress. Environ. Exp. Bot., 43: 227–237.
74. Badran, FS and Safwat MS, 2004. Response of fennel plants to organic manure and bio fertilizers in replacement of chemical fertilization. Egyptian J. Agric. Res., 82(2): 247-256.
75. Ali, AA, Ali TB and Nour KAM, 2009. Antioxidants and some natural compounds applications in relation to tomato growth, yield and chemical constituents. Annals of Agric. Sci., Moshtohor, 47 (4): 469-477.
76. Abo El-Fetooh and Hanaa A,2010. Effect of biofertilizer on the growth and chemical composition of Calendula officinalis, L. Ph.D. Thesis, Fac. Agric., Mansoura Univ., Egypt.
77. Barea, JM, Pozo MJ Azcon R, Azcon-Aguilar C, 2005. Microbial co-operation in the rhizosphere, Journal of Experimental Botany, 56: 1761-1778.

78. Chen, J. 2006. The combined use of chemical and organic fertilizers and/or bio fertilizer for crop growth and soil fertility, in: International Workshop on Sustained Management of the Soil Rhizosphere System for Efficient Crop Production and Fertilizer Use, Thailand, Oct. 16-20.
79. Zahir, ZA, Munir A, Asghar HN, Shaharoona B and Arshad M, 2008. Effectiveness of rhizobacteria containing ACC deaminase for growth promotion of peas (Pisum sativum) under drought conditions. J. Microbiol. Biotechnol.,18: 958–963.
80. Egamberdieva, D, 2011. Survival of Pseudomonas extremorientalis TSAU20 and P. chlororaphis TSAU13 in the rhizosphere of common bean (Phaseolus vulgaris) under saline conditions. Plant Soil Env., 57(3): 122–127.
81. Rojas-Tapias, D, Moreno-Galván A, Pardo-Díaz S, Obando M, Rivera, D and Bonilla R, 2012. Effect of inoculation with plant growth-promoting bacteria (PGPB) on amelioration of saline stress in maize (Zea mays). Appl Soil Ecol.doi.org/10.1016/j.apsoil.2012.01.006
82. Soliman, WS, Abdel-Haleem A, El-ShaienyH, 2014. Effect of saline water on germination and early growth stage of five Apiaceae species. Afr. J. Agric. Res., 9: 713- 719.
83. Salwa, ARH, Shaban KH, Manal A and Tantawy FM, 2010. Studies on salinity tolerance of two peanut cultivars in relation to growth, leaf water content, some chemical aspects and yield. J. Applied Sci. Res.,6: 1517-1526.
84. Ahmad, P, Hakeem KR, Kumar A, Ashraf M, Akram NA, 2012. Salt-induced changes in photosynthetic activity and oxidative defense system of three cultivars of mustard (Brassica juncea L.) Afr. J. Biotech., 11: 2694-2703.
85. Foyer, CH, 2002. The contribution of photosynthetic oxygen metabolism to oxidative stress in plants. In: Inze, D. And M. V. Montage, ed. Oxidative stress in plants. New York, USA: Taylor and Francis Publishers. pp: 33-68.
86. Khodary, SEA, 2004. Effect of salicylic acid on the growth, photosynthesis and carbohydrate metabolism in salt-stressed maize plants. Intl. J. Agri. Biol., 6:5-8.
87. Lee, G, Carrow RN and Duncan RR, 2005. Growth and water relation responses to salinity stress in halophytic seashore paspalum ecotypes. Scientia Horticulturae, 104: 221-236.
88. Zhang, J, Jia W, Yang J, Ismail AM, 2006. Role of ABA in integrating plant responses to drought and salt stresses. Field Crop Res., 97:111–119.
89. Garg, N and Manchanda G, 2009. Role of Arbuscular mycorrhizae in the alleviation of ionic, osmotic and oxidative stresses induced by salinity in Cajanus cajan (L.) Millsp. (pigeon- pea). J Agron Crop Sci., 195:110–123.
90. Rakshapal, S, Sumit KS, Rajendra PP and Alok K, 2013. Techno- logy for improving essential oil yield of Ocimum basilicum L. (sweet basil) by application of bioinoculant colonized seeds under organic field conditions. Indian Crop Prod., 45:335–342.
91. Herrera-Medina, MJ, Steinkellner S, Vierheilig H, Bote JAO, Garrido JMG, 2007. Abscisic acid determines arbuscule development and functionality in the tomato Arbuscular mycorrhizae. New Phytologist, 175:554–564.
92. Baher, ZF, Mirza M, Ghorbanli M, Rezaii MB, 2002. The influence of water stress on plant height, herbal andessential oil yield and composition in Satureja hortensis L. Flav Fragr J, 17:275-7.
93. Hendawy, SF, Khalid KA, 2005. Response of sage (Salvia officinalis L.) plants to zinc application under different salinity levels. J Appl Sci Res., 1:147-55.
94. Charles, DJ,Joly RJand Simon JE,1990. Effect of osmotic stress on the essential oil content and composition of peppermint. Phytochem., 29:2837-40.
95. Edris, AE, Ahmad S and Fadel HM, 2003. Effect of organic agriculture practices on the volatile aroma components of some essential oil plants growing in Egypt II: sweet marjoram (Origanum marjorana L.) essential oil. Flavour Fragr. J., 4: 345–51.
96. Scavroni, J, Boaro CSF, Marques MOM and Ferreira LC, 2005. Yield and composition of the essential oil of Mentha piperita L.(Lamiaceae) grown with bio solid. Brazilian J. Plant Physiol., 4: 130–45.
97. Bashan, Y., Holguin 1G and de-Bashan LE ,2004. Environmental Microbiology Group, Center for Biological Research of the Northwest (CIB), P.O. Box 128, La Paz, B.C.S. 23000, Mexico. Can. J. Microbiol., 50: 521–577.
98. Plazinski, J and Rolfe BG 1985. Influence of Azospirillum strains on the nodulation of clovers by Rhizobium strains. Appl. Environ. Microbiol., 49: 984–989.

99. Remans, R, Croonenborghs A, Torres Gutierrez R, Michiels J and Vanderleyden J, 2007. Effects of plant growth promoting rhizobacteria on nodulation of Phaseolus vulgaris L. are dependent on plant P nutrition. Eur. J. Plant Pathol.,119: 341–351.
100. Egamberdieva, D and Kucharova Z, 2009. Selection for rot colonisng bacteria stimulating wheat growth in saline soils. Biol. Fert. Soils, 45: 563–571.
101. Bianco, C. and Defez R 2009.Medicago truncatula improves salt tolerance when nodulated by an indole-3-acetic acid-overproducing Sinorhizobium meliloti strain. J. Exp. Bot., 60: 3097–3107.
102. Han, HS and Lee KD, 2005. Plant growth promoting rhizobacteria effect on antioxidant status, photosynthesis, mineral uptake and growth of lettuce under soil salinity. Res. J. Agric. Biol. Sciences, 1(3): 210–215.
103. Khalid, KA, Abou-Hussien SD, Salman SR, 2005. Influence of sulphur and biofertilizer (Sulphur-Oxidizing Bacteria) on the growth, oil and chemical composition of Celery plant. Annals of Agricultural Science,50(1): 249-262.
104. Faheed, FA, Hassanein AM and Azooz, MM, 2005. Gradual increase in NaCl concentration overcomes inhibition of seed germination due to salinity stress in Sorghum bicolor (L.) ActaAgronomica Hungarica, 53(2):229-239.
105. Yazici, I, Turkan I, Sekmen A and Demiral T, 2007. Salinity tolerance of Purslane (Portulacaolaracea L.) is achieved by enhanced ant oxidative system, lower level of lipid proxidation and proline accumulation. J. Env.Exp. Bot. A ,453: 104-112.
106. Amirjani, MR, 2010. Effect of NaCl on some hysiological parameters of rice. EKBS, 3(1):06-16.
107. Liu, J, Zhu JK.1988. A calcium homologue required for plant salt tolerance. Science, 280:1934-45.
108. Nou, X, Bressan RA, Hasegawa PM, Pardo JP, 1995. Iron homeostasis in NaCl stress environments. Plant Physiol., 109:735-742.
109. Greenway, H, Munns R, 1980. Mechanism of salt tolerance in non-halophytes. Ann Rev Plant Physiol., 31:149-190.
110. Pasternak, D, 1987. Salt tolerance and crop production, a comprehensive approach. Ann Rev Phytopathol., 25:271-291.
111. Khalil, SE, 2006. Physiological studies on sesame plants grown under saline water irrigation conditions. PhD Thesis, Faculty of Science, Cairo University, Egypt.
112. Banon, S, Ochoa J, Franco JA, Alarcon JJ and Sanchez-Blanco MJ, 2006. Hardening of oleander seedlings by deficit irrigation and low air humidity. Environ. and Exp. Bot., 56: 36-43.
113. Marulanda-Aguirre, A, Azcon R, Ruiz-Lozano JM, Aroca R, 2008. Differential effects of a Bacillus megaterium strain on Lactuca sativa plant growth depending on the origin of the arbuscular mycorrhizal fungus coinoculated: physiologic and biochemical traits. J Plant Growth Regul.,27:10–18.
114. Hashemabad, A, Zaredost F, Ziyabari MB, Zarchini M, Kaviani B, Solimandarabi MJ, Torkashvand AM, Zarchini S, 2012. Influence of phosphate bio-fertilizer on quantity and quality features of marigold (Tagetes erecta L.). Australian Journal of Crop Science, 6(6): 1101-1109.
115. Sang-Mo, MK, Ramalingam R, Young-Hyun Y, Gil-Jae J, In-Jung L, Ko-Eun L, and Jin-Ho K, 2014.Phosphate solubilizing Bacillus megaterium mj1212 regulates endogenous plant carbohydrates and amino acids contents to promote mustard plant growth. Indian J Microbiol., 54(4): 427–433.
116. Tyler, KB, May DM, Guerard D and Hatakeda JJ, 1988. Diognosing nutrient needs of garlic. USA California Agric.,116-42 (2): 28-29.
117. Talaei, GH , Eslami A, Shahghol H, 2014. Effect of biological fertilization on medicinal plants. International Journal of Biosciences | IJB |, 5(7):16-31.
118. Swedrzynska, D and Sawicka A, 2000. Effect of Inoculation with Azospirillum brasilense on Development and Yielding of Maize (Zea mays ssp. Saccharata L.) under Different Cultivation Conditions. Polish Journal of Environmental Studies, 9: 505-509.
119. Nadeem, SM, Zahir ZA, Naveed M, Arshad M, Shahzad SM, 2006. Variation in growth and ion uptake of maize due to inoculation with plant growth promoting rhizobacteria under salt stress. Soil & Environ., 25: 78-84.
120. Prathibha, KS and Siddalingeshwara KG, 2013. Effect of plant growth promoting Bacillus subtilisand Pseudomonas fluorescence as Rhizobacteria on seed quality of sorghum. IntJCur Microbiol App Sci., 2: 11-18.
121. Abdalla, MM and El-Khoshiban NH, 2007. The influence of water stress on growth, relative water content, photosynthetic pigments, some metabolic and hormonal contents of two Triticium aestivum cultuivars. Journal of Applied Sciences Research, 3(12): 2062-2074.

122. Al-Amier, H and Craker LE, 2007. In-Vitro selection for stress tolerant spearmint. Reprinted from: Issues in new crops and new uses. J. Janick and A. Whipkey (eds.). ASHS Press, Alexandria, VA. 306-310.
123. Zaki, MF, Abou-Hussein SD, Abou El-Magd MM and El-Abagy HMH, 2009. Evaluation of some sweet fennel cultivars under saline irrigation water. Eur J Sci Res., 30(1):67-78.
124. Najafi, F, Khavari-Nejad RA and Ali MS 2010. The effects of salt stress on certain physiological parameters in summer savory (Satureja hortensis L.) plants. J Stress Physiol Biochem, 6(1):13-21.
125. Ashraf, M and Orooj A, 2006. Salt stress effects on growth, ion accumulation and seed oil concentration in an arid zone traditional medicinal plant ajwain (Trachyspermum ammi [L.] Sprague). J Arid Environ., 64(2):209-20.
126. Osman, MEH, Elfeky SS, Abo El-Soud K and Hasan AM, 2007. Response of Catharanthus roseus shoots to salinity and drought in relation to vincristine alkaloid content. Asian J Plant Sci., 6:1223-1228.
127. Cik, JK, Klejdus B, Hedbavny J, Bačkor M, 2009. Salicylic acid alleviates NaCl induced changes in the metabolism of Matricaria chamomilla plants. Ecotoxicology, 18(5):544-54.
128. Muthukumarasamy M, Gupta DS and Panneerselvam R, 2000. Influence of triadimefon on the metabolism of NaCl stressed radish. Biol Plant, 43:67-72.
129. Levitt, J,1980. Responses of plants to environmental stresses, Academic Press, Vol. II, New York.
130. Hasogawa, PM, Bresson RA and Handa AV, 1986. Cellular mechanisms of salinity tolerance. Hort. Science, 21:1317-1324.
131. Hathout, TA, 1996. Salinity stress and its concentration by the growth regulator in wheat plant. Egyption J. Physiological Sci., 20: 127-152.
132. Hafsa, CS, Allaoua S, Mostefa G, Bilal Y and Fouzia A, 2013. Solubilization of phosphate by the Bacillus under salt stress and in the presence of osmo protectant compounds. African Journal of Microbiology Research, 7(37): 4562-4571.
133. Naz, I, Bano A and Tamoor-ul-Hassan, 2009. Isolation of phytohormones producing plant growth promoting rhizobacteria from weeds growing in Khewra salt range, Pakistan and their implication in providing salt tolerance to Glycine max L. Afr. J. Biotechnol., 8(21):5762–5766.
134. Bano, A and Fatima M, 2009. Salt tolerance in Zea mays (L.) following inoculation with Rhizobium and Pseudomonas. Biol. Fertility Soils, 45:405–413.
135. Yao, L, Wu Z, Zheng Y, Kaleem I and Li C, 2010. Growth promotion and protection against salt stress by Pseudomonas putida Rs-198 on cotton. Eur. J. Soil Biol., 46:49–54.
136. Chen, M, Wei H, Cao J, Liu R, Wang Y and Zheng C, 2007. Expression of Bacillus subtilis proAB genes and reduction of feedback inhibition of proline synthesis increases proline production and confers osmotolerance in transgenic Arabdopsis. J. Biochem. Mol. Biol., 40(3):396–403.
137. Upadhyay, SK, Singh JS, Saxena AK and Singh DP, 2011. Impact of PGPR inoculation on growth and antioxidant status of wheat under saline conditions. Plant Biol.,14:605–611.

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