RESPONSE OF *THYMUS VULGARIS* L. PLANT GROWN IN SANDY SOIL TO BIOFERTILIZATION UNDER DRIPPING IRRIGATION SYSTEM

Abd El-Gawad, A.M. and W.M. Abd El Azim *
Soil Fertility and Microbiology Department, Desert Research Center El-Matariya, Cairo, Egypt.
* Medicinal and Aromatic Plants Department, Desert Research Center El-Matariya, Cairo, Egypt.

Two successive field experiments were carried out at El-Maghara Research Station in middle Sinai which belongs to Desert Research Center (DRC) during 2003-2004 and 2004-2005 seasons to study the effect of biofertilization with *Azotobacter chroococcum* and *Bacillus megatherium* as Nitrogen fixing and phosphate dissolving bacteria, respectively, on the growth, yield and essential oil proportion of *Thymus vulgaris* L. plant grown in sandy soil using dripping irrigation system. They were applied by three methods: soil drench, foliar spray and both soil drench plus foliar spray (10^8 cfu /ml).

The obtained data revealed that the best yields of the essential oil per feddan, plant yield and total microbiological counts were obtained applying combinations of both bacterial isolates as soil drench plus foliar spray method. Applying *A. chroococcum* and *B. megatherium* as foliar spray and soil drench reported the highest total microbial counts. On the other hand, phosphate solubilization by *B. megatherium* inoculation was more effective in increasing phosphate solubilization than inoculation with *A. chroococcum.* Finally, the application of biofertilizers increased the antagonistic activity of *T. vulgaris* essential oil against some tested pathogenic microbes.

Keywords: *Azotobacter chroococcum*, *Bacillus megatherium*, phosphate dissolving bacteria, sandy soil, *Thymus vulgaris*, biofertilization, antimicrobial activity.

Thyme (*Thymus vulgaris* L.) belongs to the family of Lamiaceae distributed in different areas of the Mediterranean Sea, Asia and Central and Eastern Europe (Shalaby and Razin, 1992; Badi *et al.*, 2004). Herbal medicine *T. vulgaris* is prescribed oral for dry cough, laryngitis, bronchitis, asthma, pulmary infection and chronic gastritis. Moreover, the herb is applied

externally against fungal infections, rheumatism, arthritis, tonsillitis and gum infections (Blanco *et al.*, 1998).

Many authors reported the significant effects of biofertilizers on the growth of several plants, e.g. *Mentha viridis* L. (Attia and Hoda, 2004) and *Salvia officinalis* (Youssef *et al.*, 2004). Bacterial fertilizers are preparations of living bacteria, which are applied to seeds, roots or soils to improve plant growth parameters and crop yield. However, *Azotobacter* species might be more effective when combined with other bacterial fertilizers, particularly *Bacillus megatherium,* so inoculation with a bacterial mixture could improve crop yield (Brown *et al.,* 1964; Reynders and Vlassak, 1982).

Moreover, *Azotobacter* species are able to improve the nitrogen uptake by plants through nitrogen fixation and also to synthesize biologically active compounds such as vitamins, gibberellins, nicotinic acid, panthenic acid, biotin, heteroauxin and other compounds which stimulate the growth and yield of plants and are also able to produce fungistatical substances (conactin group) inhibiting the growth of some plant .pathogenic fungi (El-Shazly, 2003; Revillas *et al.,* 2005). On the other hand, phosphate dissolving bacteria have the ability to secrete a phosphatase enzyme, which transforms organic phosphate into inorganic phosphate compounds to be available for plant uptake (Abd El-Gawad, 1999; Khan *et al.*, 2006).

The aim of this study is to investigate the effect of specific biofertilizers on the development of the thyme plant grown under sandy soil conditions of El-Maghara Research Station. The study included antagonistic effects of thyme essential oil against some pathogenic microorganisms.

MATERIALS AND METHODS

Evaluation of Biofertilizer Applications in the Field

A field experiment was established at El-Maghara Research Station of Desert Research Center (DRC) during two successive seasons, 2003-2004, and 2004-2005 to study the effect of *Azotobacter chroococcum* and *Bacillus megatherium* var. *phosphaticum* as biofertilizers on the growth of thyme plant in sandy soils.

Bacterial Culture Preparation

The systematic biotechnology was used taking fresh liquid cultures 48 hrs old from pure local strains of *A. chroococcum* and *B. megatherium* var. *phosphaticum,* previously isolated from the rhizosphere of the soils at El-Maghara area, purified and identified according to Bergey's Manual (1984), as biofertilizers in the form of single and mixed inoculations at the rate of $\sim 10^8$ cfu/ml.

Application Methods

Bacterial strains were applied separately or in combination as soil drench and/or foliar spray treatment. Five months old thyme seedlings were

soaked in a single or mixture of bacterial suspensions (10^8 cfu/ml) for 3hrs before transplanting (carboxy methyl cellulose 0.5% was used as an adhesive agent). Control plants were soaked in water only.

1 - for soil drench

Bacterial suspensions (10^8 cfu/ml) were applied as drench to the soil around seedlings at planting time. An additional water treatment was preformed as a control.

2 - for foliar spray treatment

Bacterial suspensions (10^8 cfu/ml) were applied as foliar spray over seedlings at planting time. A water spray treatment was preformed as a control.

Twenty one days later bacterial suspensions were applied once again for both foliar spray and soil drench treatments. This experiment included nine treatments within a split plot design; the unit area was 20 m^2. Each unit included three rows, each row was 20 m in length and 100 cm width. The physical and chemical analyses of soil, irrigation water and sheep manure are presented in tables (1 – 4). Soil analyses were carried out at the Soil Analysis Laboratory of DRC. During the growing season, congenital cultural practices were conducted where experimental plots were irrigated using a drip irrigation system for 1/2 h/day. Also, sheep manure of 20 m^3/feddan as organic manure was provided with 31 kg P_2O_5/feddan, mixed with the soil before sowing, N and K fertilizers were added at rate of 60 kg N/fed. as NH_4NO_3 and 75 kg K_2O/fed. as K_2SO_4 into three equal doses.

Table (1). Particles size distribution of the experimental soil.

Very coarse sand (%) (2:1 mm)	Coarse sand (%) (1:0.5mm)	Medium sand (%) (0.5:0.25mm)	Fine sand (%) (0.25:0.1mm)	Very fine sand (%) (0.1:0.063mm)	Silt and clay (%) (<0.063mm)	Soil texture
1.27	5.90	15.30	61.28	12.82	3.43	Sandy

Table (2). Chemical properties of the experimental soil.

pH	E.C. (dSm^{-1})	O.M. (%)	Soluble cations (meq./l)				Soluble anions (meq./l)			
			K^+	Na^+	Mg^{++}	Ca^{++}	CO_3^{--}	HCO_3^-	Cl^-	SO_4^{--}
8.70	0.67	0.47	0.09	2.43	0.80	3.20	-	3.00	1.38	2.14

Table (3). Irrigation water analysis.

pH	E.C. (dSm^{-1})	O.M. (%)	Soluble cations (meq./l)				Soluble anions (meq./l)			
			K^+	Na^+	Mg^{++}	Ca^{++}	CO_3^{--}	HCO_3^-	Cl^-	SO_4^{--}
8.36	4.06	0.4	0.69	24.60	3.48	11.40	-	4.40	32.20	3.57

Table (4). Sheep manure analysis.

O.C. %	N %	C/N	P	K	Fe	Mn	Zn	Cu	pH
			ppm						
20.1	1.6	12.56	22	128	356	59	15	7.9	7.5

O.C. organic carbon

Plant Growth Parameters

The harvested cuts were taken in June and September in the both seasons. Meanwhile, fresh and dry weights (g/plant), dry weight (kg/feddan) and oil yield (L /feddan) were recorded at each cut.

Chemical Analysis

The chemical analysis included chlorophyll a and b and carrotenoids according to Cherry (1973). The highest percentage of essential oil in different treatments and the control was determined using GLC analysis (apparatus model PRO-GC Pye Unican Philips with Column PEGA 10%). The total nitrogen was also determined according to a modified Kjeldahl method as described by Allen (1959).

Total Count of Soil Microorganisms

Soil samples of the *T. vulgaris* rhizosphere were collected at the end of the first and second cut in both seasons and analyzed for total count of microorganisms according to Bunt and Rovira (1955) as follows:

a- For counting and growing phosphate dissolving bacteria, the same medium was used after addition of 5 ml sterile solution of 10 % of K_2HPO_4 and of 10 ml of sterile solution of 10% $CaC1_2$ to each 100 ml of the medium (Abd El-Hafez, 1966).

b- For counting and growing azotobacters, nitrogen deficient medium was used as described by Abd El-Malek and Ishac (1968).

Antimicrobial Activity

The antimicrobial activity of *T. vulgaris* essential oil was proved against some pathogenic microorganisms, namely, *Bacillus subtilis, Staphylococcus aureus, Candida albicans, Salmonella typhi, Escherichia coli, Rhizoctonia solani* and *Aspergillus albus.* These microorganisms were provided by the Animal Health Department and Plant Pathology Unit, DRC. The antimicrobial activity was determined by the agar diffusion technique using filter paper discs according to the method of Maruzzella and Balter (1959). Culture medium was prepared using nutrient broth and nutrient agar medium according to the method of Waksman and Lechevalier (1962).

Statistical Aanalysis

Data were analysed according to the procedure analysis of variance "Anova" reported by Snedecor and Cochran (1982). Treatment means were compared by the Duncan's multiple Range Test at 5% level of probability.

RESULTS AND DISCUSSION

Effect of Biofertilizers Applied as Foliar Spray and/or Soil Drench Treatments on the Plant Growth Character

Data in tables (5, 6 and 7) show that thyme seedlings treated with *A. chroococcum* and *B. megatherium* as individual or in mixture using various application methods like foliar spray and/or soil drench possessed

significantly higher amounts of fresh, dry weights and dry yield as compared to untreated plants. The highest fresh weight/plant and dry yield/feddan were gained when biofertilizers were applied in combination. The differences within treatments may be related to either the variation of nutrient accumulations, or to the type and nature of growth co-factors variation due to biofertilization. Both nutrients and growth co-factors varied within the used biofertilizer organisms, these variations depended upon the prevailing environmental conditions (Holla and Vaverkova, 1993) and on cuts. The plant age as well may play a role in this respect.

Table (5). Effect of biofertilizers applied as foliar spray (FS) and/or soil drench (SD) treatments on thyme plant fresh weight (g).

First season (2003-2004)										
Treatment	**First cut**					**Second cut**				
Fertilizer / Methods	**control**	***Azoto.***	***Bacil.***	***Azoto+ Bacil.***	**mean**	**control**	***Azoto.***	***Bacil.***	***Azoto+ Bacil.***	**mean**
FS	9.53	16.77	15.47	17.60	14.84	53.40	83.12	67.55	85.50	72.39
SD	8.97	20.17	18.88	20.50	17.13	64.40	99.40	88.35	112.83	91.25
FS +SD	10.53	20.33	19.90	20.63	17.85	61.37	101.33	91.73	121.00	93.86
Mean	9.68	19.09	18.08	19.58	16.61	59.72	94.62	82.54	106.44	85.83
L.S.D. P≤ 0.05	Methods 0.75 Fertilizer 1.09 Methods *fertilizer 1.67					Methods 3.00 Fertilizer 1.87 Methods *fertilizer 3.24				
Second season (2003-2004)										
Treatment	**First cut**					**Second cut**				
Fertilizer / Methods	**control**	***Azoto.***	***Bacil.***	***Azoto+ Bacil.***	**mean**	**control**	***Azoto.***	***Bacil.***	***Azoto+ Bacil.***	**mean**
FS	10.97	32.38	27.69	32.84	25.97	43.68	83.32	64.51	96.36	71.97
SD	11.53	44.54	37.79	51.00	36.22	48.97	101.12	96.81	138.34	96.31
FS +SD	12.53	49.33	41.04	56.71	39.90	53.19	111.63	99.53	139.61	100.99
Mean	11.68	42.08	35.51	46.85	34.03	48.61	98.69	86.95	124.77	89.76
L.S.D. P≤ 0.05	Methods 5.70 Fertilizer 5.00 Methods *fertilizer 8.67					Methods 1.78 Fertilizer 1.85 Methods *fertilizer 3.19				

Azoto.=Azotobacter chroococcum , Bacil=Bacillus megatherium.

Table (6) Effect of biofertilizers applied as foliar spray (FS) and/or soil drench (SD) treatments on thyme plant dry weight (g).

First season (2003-2004)										
Treatment	First cut					Second cut				
Fertilizer / Methods	control	*Azoto.*	*Bacil.*	*Azoto+ Bacil.*	mean	control	*Azoto.*	*Bacil.*	*Azoto+ Bacil.*	mean
FS	3.50	5.40	4.60	5.49	4.75	18.40	25.10	20.41	25.77	22.42
SD	2.10	6.39	5.93	7.04	5.37	21.97	29.98	23.10	37.16	28.05
FS +SD	3.63	6.65	5.98	7.12	5.85	18.70	30.66	27.78	37.64	28.70
Mean	3.08	6.15	5.50	6.55	5.32	19.69	28.58	23.76	33.52	26.39
L.S.D. P≤ 0.05	Methods 0.08 Fertilizer 0.16 Methods *fertilizer 0.09					Methods 0.81 Fertilizer 1.11 Methods *fertilizer 1.92				
Second season (2003-2004)										
Treatment	First cut					Second cut				
Fertilizer / Methods	control	*Azoto.*	*Bacil.*	*Azoto+ Bacil.*	mean	control	*Azoto.*	*Bacil.*	*Azoto+ Bacil.*	mean
FS	4.03	15.31	11.85	13.64	11.21	15.05	26.59	22.77	35.09	24.88
SD	2.90	17.97	14.35	19.86	13.77	16.70	38.23	29.80	53.74	34.62
FS +SD	4.30	18.45	17.32	21.11	15.30	16.21	42.05	35.37	54.08	36.93
Mean	3.74	17.24	14.51	18.20	13.42	•15.99	35.62	29.31	47.64	32.14
L.S.D. P≤ 0.05	Methods 0.30 Fertilizer 0.69 Methods *fertilizer 1.19					Methods 0.49 Fertilizer 0.69 Methods *fertilizer 1.21				

Azoto.= Azotobacter chroococcum , Bacil=Bacillus megatherium

Table (7). Effect of biofertilizers applied as foliar spray (FS) and/or soil drench (SD) treatments on thyme plant dry weight (kg/feddan).

First season (2003-2004)										
Treatment	First cut					Second cut				
Fertilizer / Methods	control	*Azoto.*	*Bacil.*	*Azoto+ Bacil.*	mean	control	*Azoto.*	*Bacil.*	*Azoto+ Bacil.*	mean
FS	29.40	45.36	38.64	46.14	39.89	154.56	210.81	171.47	216.50	188.34
SD	17.64	53.68	49.84	59.14	45.08	184.55	251.83	194.70	312.12	235.80
FS +SD	30.52	55.86	50.20	59.84	49.11	157.08	257.57	233.35	316.20	241.05
Mean	25.85	51.63	46.23	55.04	44.69	165.40	240.07	199.84	281.61	221.73
L.S.D. P≤ 0.05	Methods 0.66 Fertilizer 1.33 Methods *fertilizer 2.31					Methods 6.77 Fertilizer 9.29 Methods *fertilizer 16.09				
Second season (2003-2004)										
Treatment	First cut					Second cut				
Fertilizer / Methods	control	*Azoto.*	*Bacil.*	*Azoto+ Bacil.*	mean	control	*Azoto.*	*Bacil.*	*Azoto+ Bacil.*	mean
FS	33.82	128.58	99.57	114.55	94.13	126.42	223.36	191.24	294.73	208.94
SD	24.33	150.98	120.57	166.85	115.68	140.31	321.10	250.32	451.44	290.79
FS +SD	36.12	154.98	145.46	177.30	128.47	136.16	353.22	297.11	454.27	310.19
Mean	31.42	144.85	121.87	152.90	112.76	134.30	299.23	246.22	400.15	269.97
L.S.D. P≤ 0.05	Methods 2.56 Fertilizer 5.78 Methods *fertilizer 10.00					Methods 4.13 Fertilizer 5.87 Methods *fertilizer 10.17				

Azoto.= Azotobacter chroococcum , Bacil=Bacillus megatherium

Photosynthetic Pigments

Data presented in table (8) show that the highest photosynthetic pigment concentrations were recorded in the mixed treatment with foliar spray in addition to soil drench. The results revealed that:

a) Different treatments not only affected photosynthetic pigment concentrations in leaves of the thyme plant, but also regulated the ratio between the chlorophyll A; chlorophyll B and the total chlorophylls and carrotenoids.

b) The time of cut collection seemed to influence the photosynthetic pigments and in general environmental conditions play a role in photosynthetic regulations.

Table (8). Effect of biofertilizers applied as foliar spray (FS) and/or soil drench (SD) treatments on photosynthetic pigments (mg/g fresh weight) of thyme plants.

First season (2003-2004)									
Treatment		First cut				Second cut			
Fertilizer / Methods	Chlorophyll	control	*Azoto.*	*Bacil*	*Azoto+ Bacil.*	control	*Azoto.*	*Bacil.*	*Azoto+ Bacil.*
FS	A	0.427	0.525	0.507	0.602	0.485	0.601	0.600	0.679
	B	0.231	0.278	0.238	0.298	0.290	0.372	0.365	0.406
	Total A+B	0.658	0.803	0.745	0.900	0.775	0.973	0.965	1.085
	Carrotenoid	0.144	0.162	0.166	0.167	0.154	0.173	0.171	0.187
SD	A	0.420	0.557	0.539	0.639	0.422	0.686	0.614	0.702
	B	0.232	0.250	0.334	0.368	0.250	0.422	0.355	0.430
	Total A+B	0.652	0.807	0.873	0.1007	0.672	1.108	0.969	1.132
	Carrotenoid	0.135	0.160	0.175	0.180	0.149	0.183	0.164	0.173
FS +SD	A	0.482	0.696	0.643	0.702	0.546	0.811	0.681	0.956
	B	0.228	0.349	0.280	0.361	0.336	0.443	0.401	0.542
	Total A+B	0.710	1.045	0.923	1.063	0.882	1.254	1.082	1.498
	Carrotenoid	0.142	0.209	0.200	0.181	0.147	0.239	0.177	0.203
Second season (2003-2004)									
Tretmeant		First cut				Second cut			
Fertilizer / Methods	Chlorophyll	control	*Azoto.*	*Bacil*	*Azoto+ Bacil.*	control	*Azoto.*	*Bacil*	*Azoto+ Bacil.*
FS	A	0.414	0.577	0.569	0.583	0.567	0.606	0.601	0.644
	B	0.224	0.263	0.314	0.255	0.335	0.370	0.355	0.365
	Total A+B	0.638	0.840	0.883	0.838	0.902	0.976	0.956	1.009
	Carrotenoid	0.139	0.167	0.193	0.163	0.162	0.160	0.212	0.167
SD	A	0.502	0.602	0.598	0.652	0.598	0.698	0.643	0.735
	B	0.237	0.317	0.279	0.323	0.352	0.428	0.392	0.404
	Total A+B	0.739	0.919	0.877	0.975	0.950	1.126	1.035	1.139
	Carrotenoid	0.146	0.183	0.195	0.183	0.167	0.171	0.164	0.217
FS +SD	A	0.483	0.697	0.639	0.709	0.583	0.749	0.700	0.845
	B	0.290	0.366	0.319	0.403	0.359	0.442	0.411	0.486
	Total A+B	0.773	1.063	0.958	1.112	0.942	1.191	1.111	1.331
	Carrotenoid	0.159	0.176	0.189	0.199	0.167	0.235	0.181	0.182

Azoto.= Azotobacter chroococcum , Bacil=Bacillus megatherium

Effect of Biofertilizers Applied as Foliar Spray and/or Soil Drench Treatments on the Essential Oil of Dry Shoots of the Thyme Plants

Data in table (9) apparently show that applying biofertilizers as foliar spray and/or soil drench affected significantly the percentage of the essential oil in dry shoots of the thyme plant. This detection appeared within the two

cuts and during both annual trials. All treatments increased such proportion over the control plants. The highest essential oil seemed to be found in those plants supplied with a mixed inoculation as foliar plus soil drench application, which is considered as the best method of application. The combined application of both biofertilizer organisms proved to be better than the single application. The combination might have some stimulating effects on the proportion of essential oil in the shoots dry matter. The harvesting time of dry matter and the prevailing environmental conditions seemed to be without clear effects in this respects.

Table (9). Effect of biofertilizers applied as foliar spray (FS) and/or soil drench (SD) treatments on oil dry shoots (%) of thyme plants.

First season (2003-2004)										
Treatment	First cut					Second cut				
Fertilizer / Methods	control	*Azoto.*	*Bacil.*	*Azoto+ Bacil.*	mean	control	*Azoto.*	*Bacil.*	*Azoto+ Bacil.*	mean
FS	1.75	1.90	2.27	2.60	2.13	1.80	2.10	2.40	2.58	2.22
SD	1.75	2.08	2.30	2.76	2.22	1.60	2.15	2.45	2.70	2.23
FS +SD	2.00	2.25	2.40	2.80	2.36	2.00	2.23	2.48	2.75	2.37
Mean	1.83	2.08	2.32	2.72	2.24	1.80	2.16	2.44	2.68	2.27
L.S.D. P≤ 0.05	Methods 0.05 Fertilizer 0.06 Methods *fertilizer 0.10					Methods 0.14 Fertilizer 0.13 Methods *fertilizer 0.23				
Second season (2003-2004)										
Treatment	First cut					Second cut				
Fertilizer / Methods	control	*Azoto.*	*Bacil.*	*Azoto+ Bacil.*	mean	control	*Azoto.*	*Bacil.*	*Azoto+ Bacil.*	mean
FS	1.70	2.00	2.30	2.67	2.17	1.65	1.95	2.38	2.58	2.14
SD	1.80	2.13	2.35	2.70	2.25	1.70	2.15	2.50	2.65	2.25
FS +SD	1.90	2.32	2.53	2.75	2.38	1.83	2.20	2.58	2.73	2.34
Mean	1.80	2.15	2.39	2.71	2.26	1.73	2.10	2.49	2.65	2.24
L.S.D. P≤ 0.05	Methods 0.13 Fertilizer 0.15 Methods *fertilizer 0.26					Methods 0.14 Fertilizer 0.16 Methods *fertilizer 0.28				

Azoto.= Azotobacter chroococcum , Bacil=Bacillus megatherium

Effect of Biofertilizers Applied as Foliar Spray or/and Soil Drench Treatments on the Yield of Thyme Essential Oil (l/feddan)

Data in table (10) revealed that the different tested factors like the species of bacteria, application method and time of collecting cuts seemed to have a role on the yielded essential oil. The essential oil of thyme plants was always higher in the second cut than in the first one. The essential oil yield/feddan was higher during the second season than those corresponding ones of the first season. These data may indicate that the environmental conditions probably have a role on the yielded oil productivity. The highest yield of oil was gained by using both agents of biofertilizers, *A. chroococcum* + *B. megatherium*. The best method of biofertilization is a mixed inoculation with foliar and soil drench application.

Gas Liquid Chromatography (GLC) Analysis of Thyme Plants Essential Oil (%) as Affected by Biofertilizers Applied as Foliar Spray and/or Soil Drench Treatments

Data in table (11) indicated that thymol is the main dominant fraction in essential oil of thyme plants treated with a single or with a mixture of both biofertilizer as foliar spray and/or soil drench treatment compared with the control, followed by p-cymene, 1-8 cineol and conlrile. Whereas, α-pinene achieved the lowest quantity. Different treatments affected the distribution of different essential oil fractions.

Table (10). Effect of biofertilizers applied as foliar spray (FS) and/or soil drench (SD) treatments on thyme oil yield (L/feddan).

First season (2003-2004)										
Treatment	First cut					Second cut				
Fertilizer / Methods	control	*Azoto.*	*Bacil.*	*Azoto+ Bacil.*	mean	control	*Azoto.*	*Bacil.*	*Azoto+ Bacil.*	mean
FS	0.51	0.86	0.88	1.20	0.86	2.78	4.43	4.12	5.59	4.23
SD	0.31	1.12	1.15	1.63	1.05	2.95	5.41	4.77	8.43	5.39
FS +SD	0.61	1.26	1.20	1.68	1.19	3.14	5.74	5.79	8.70	5.84
Mean	0.48	1.08	1.08	1.50	1.03	2.96	5.20	4.89	7.57	5.15
L.S.D. P≤ 0.05	Methods 0.02 Fertilizer 0.03 Methods *fertilizer 0.05					Methods 0.15 Fertilizer 0.19 Methods *fertilizer 0.34				
Second season (2003-2004)										
Treatment	First cut					Second cut				
Fertilizer / Methods	control	*Azoto.*	*Bacil.*	*Azoto+ Bacil.*	mean	control	*Azoto.*	*Bacil.*	*Azoto+ Bacil.*	mean
FS	0.57	2.57	2.29	3.06	2.12	2.09	4.36	4.55	7.60	4.65
SD	0.44	3.22	2.83	4.50	2.75	2.39	6.90	6.26	11.96	6.88
FS +SD	0.69	3.60	3.68	4.88	3.21	2.49	7.77	7.67	12.40	7.58
Mean	0.57	3.13	2.93	4.15	2.69	2.32	6.34	6.16	10.66	6.37
L.S.D. P≤ 0.05	Methods 0.05 Fertilizer 0.15 Methods *fertilizer 0.26					Methods 0.09 Fertilizer 0.14 Methods *fertilizer 0.25				

Azoto.= Azotobacter chroococcum , Bacil=Bacillus megatherium

Table (11) Effect of biofertilizers applied as foliar spray (FS) and/or soil drench (SD) treatments on percentage of thyme oil constituents.

Oil	FS+SD control %	SD *Azoto+Bacil* %	FS +SD *Azoto+Bacil* %
α-pinene	0.242	0.347	0.619
β-pinene	1.488	1.507	1.795
α-terpineol	1.049	1.085	1.433
p-cymene	15.245	19.656	19.698
1-8 cineol	4.821	5.755	6.947
linalol	1.933	2.840	3.077
borneol	0.814	0.884	2.082
thymol	49.562	49.803	51.447
carvacrol	3.727	4.315	6.638
eugenol	-	-	0.301

Effect of Biofertilizers Applied as Foliar Spray and/or Soil Drench Treatments on Total Count of Soil Microorganisms (cfu/g)

a - Total microbial count

Initial total microbial count in El-Maghara soil was 13×10^5 cfu/g dry soil. Results in table (12) show the change in count which tend to increase in all treatments compared to the control. The total microbial count proved an increase in the second cut during the first and second seasons. A mixed inoculation with *A. chroococcum* and *B. megatherium* produced the highest increase in the total microbial count. Similarly, Subba Rao (1988) and Abd El-Ghany *et al.* (1997) reported that microbial inoculants increase the number and biological activities of desired microorganisms and improve the fertility in the root zone.

Table (12). Effect of biofertilizers applied as foliar spray (FS) and/or soil drench (SD) treatments on the total microbial count (10^6 cfu/g dry soil).

First season (2003-2004)										
Treatment	First cut					Second cut				
Fertilizer / Methods	control	*Azoto.*	*Bacil.*	*Azoto+ Bacil.*	mean	control	*Azoto.*	*Bacil.*	*Azoto+ Bacil.*	mean
FS	12.45	20.10	19.60	22.75	18.73	13.15	26.70	22.34	25.15	21.86
SD	12.71	24.80	20.95	26.90	21.34	13.90	29.20	24.19	28.20	23.87
FS +SD	12.85	26.95	22.80	29.50	23.03	14.21	29.45	26.50	33.10	25.82
Mean	12.67	23.95	21.12	26.38	21.03	13.75	28.48	24.34	28.83	23.84
L.S.D. P≤ 0.05	Methods 0.059 Fertilizer 0.060 Methods *fertilizer 0.119					Methods 0.060 Fertilizer 0.070 Methods *fertilizer 0.120				
Second season (2003-2004))										
Treatment	First cut					Second cut				
Fertilizer / Methods	control	*Azoto.*	*Bacil.*	*Azoto+ Bacil.*	mean	control	*Azoto.*	*Bacil.*	*Azoto+ Bacil.*	mean
FS	13.61	22.40	21.60	24.35	20.49	14.00	29.50	27.50	29.10	25.03
SD	13.86	26.81	24.35	27.40	23.11	14.36	33.90	28.50	36.10	28.22
FS +SD	14.11	27.71	25.90	28.64	24.09	14.95	36.50	31.50	39.10	30.51
Mean	13.86	25.64	23.95	26.80	22.56	14.43	33.30	29.17	34.77	27.92
L.S.D. P≤ 0.05	Methods 0.050 Fertilizer 0.060 Methods *fertilizer 0.082					Methods 0.085 Fertilizer 0.070 Methods *fertilizer 0.128				

Azoto.= Azotobacter chroococcum , Bacil=Bacillus megatherium/ c.f.u.= colony forming unit

b - *Azotobacter* densities

The initial count of N_2 fixing azotobacters in El-Maghara soil was 10×10^4 cfu/g dry soil. Data recorded in table (13) show that the count reported a marked increase in the first cut and increase gradually in the second cut in the first season. The same trend was recorded in the second season. The counts under *A. chroococcum* inoculation showed the highest

counts all over the experimental periods while PDB (phosphate dissolving bacteria) inoculation caused the least increase of azotobacters count. Also, mixed applications of *A. chroococcum*+ *B. megatherium* (foliar spray+ soil drench) reported the highest counts. The obtained results proved that N_2 fixers *A. chroococcum* enrich the soil by nitrogen fixation which increase soil fertility. The promoting effect due to application of *A. chroococcum* is not only due to the nitrogen fixation but also to the production of plant growth promoting substances, production of amino acids, organic acids, vitamins and antimicrobial substances as well, which increase soil fertility, microbial community and plant growth (Revillas *et al.,* 2005).

Table (13) Effect of biofertilizers applied as foliar spray (FS) and/or soil drench (SD) treatments on the *Azotobacter* counts (counts × 10^5 cfu/g dry soil).

First season (2003-2004)										
Treatment	First cut					Second cut				
Fertilizer / Methods	control	*Azoto.*	*Bacil.*	*Azoto+ Bacil.*	mean	control	*Azoto.*	*Bacil.*	*Azoto+ Bacil.*	mean
FS	10.1	13.2	11.8	18.0	13.25	12.2	14.3	12.5	27.2	16.6
SD	10.9	23.0	11.8	28.6	18.6	12.3	37.0	12.9	41.0	25.8
FS +SD	10.7	24.5	13.2	29.4	19.5	12.6	38.2	13.9	47.6	28.1
Mean	10.6	20.2	12.3	25.3	17.1	12.4	29.8	13.1	38.6	23.5
L.S.D. P≤ 0.05	Methods 0.350 Fertilizer 0.400 Methods *fertilizer 0.175					Methods 0.250 Fertilizer 0.300 Methods *fertilizer 0.095				
Second season (2003-2004)										
Treatment	First cut					Second cut				
Fertilizer / Methods	control	*Azoto.*	*Bacil.*	*Azoto+ Bacil.*	mean	control	*Azoto.*	*Bacil.*	*Azoto+ Bacil.*	mean
FS	11.40	13.6	11.9	13.8	12.6	13.1	14.6	14.5	15.0	14.3
SD	12.1	27.3	12.1	28.1	19.9	13.6	42.5	14.1	44.3	28.6
FS +SD	12.5	29.4	12.7	31.1	21.25	14.1	46.1	14.8	49.8	31.2
Mean	12	23.4	12.23	24.3	26.6	13.6	34.4	14.5	36.4	24.7
L.S.D. P≤ 0.05	Methods 0.080 Fertilizer 0.097 Methods *fertilizer 0.240					Methods 0.256 Fertilizer 0.296 Methods *fertilizer 0.220				

Azoto.= Azotobacter chroococcum , *Bacil=Bacillus megatherium* , c.f.u.= colony forming unit

c - Phosphate dissolving bacterial count

Data in table (14) reveal that, the counts of *B. megatherium* under inoculation with the same organism showed the highest counts all over the experimental periods. Also, a mixed application of *B. megatherium* + *A. chroococcum* applied as foliar spray and soil drench reported the highest count. It is worthy to notice that the initial count of phosphate dissolving bacteria *B. megatherium* in El-Maghara soil was 2.5×10^3 cfu/g of dry soil. *Bacillus. megatherium* inoculation stimulated the organism and increased its density compared to other treatments.

Table (14). Effect of biofertilizers applied as foliar spray (FS) and/or soil drench (SD) treatments on the *Bacillus megaterium* count (10^2 cfu/g dry soil).

First season (2003-2004)										
Treatment	First cut					Second cut				
Fertilizer / Methods	control	*Azoto.*	*Bacil.*	*Azoto+ Bacil.*	mean	control	*Azoto.*	*Bacil.*	*Azoto+ Bacil.*	mean
FS	2..55	6.01	10.80	11.17	7.63	2.81	6.24	11.20	11.43	7.89
SD	2.61	6.04	11.60	11.90	8.01	2.72	6.32	12.50	12.92	8.63
FS +SD	2.84	6.14	11.80	12.12	8.22	2.91	6.39	12.90	13.10	8.83
Mean	2.67	6.06	11.40	11.73	7.97	2.81	6.32	12.20	12.48	8.45
L.S.D. P≤ 0.05	Methods 0.049 Fertilizer 0.057 Methods *fertilizer 0.181					Methods 0.049 Fertilizer 0.057 Methods *fertilizer 0.680				
Second season (2003-2004)										
Treatment	First cut					Second cut				
Fertilizer / Methods	control	*Azoto.*	*Bacil.*	*Azoto+ Bacil.*	mean	control	*Azoto.*	*Bacil.*	*Azoto+ Bacil.*	mean
FS	2.70	6.35	11.21	11.42	7.92	2.89	6.70	11.84	11.91	8.33
SD	2.75	6.49	12.05	12.45	8.44	2.94	7.01	12.84	12.97	8.94
FS +SD	2.86	6.62	12.37	12.80	8.66	3.10	7.21	13.10	13.70	9.28
Mean	2.77	6.49	11.88	12.22	8.34	2.98	6.97	12.59	12.86	8.85
L.S.D. P≤ 0.05	Methods 0.040 Fertilizer 0.040 Methods *fertilizer 0.320					Methods 0.026 Fertilizer 0.296 Methods *fertilizer 0.177				

Azoto.= Azotobacter chroococcum , Bacil=Bacillus megatherium , c.f.u.= colony forming unit

d - Activities of phosphate dissolving bacteria

Table (15) show that phosphate solubilization by *B. megatherium* inoculation was more effective in phosphate solubilization than the *A. chroococcum* inoculation (due to production of organic acids). The maximum phosphate solubilization activity was recorded with a mixed treatment (*A. chroococcum* + *B. megatherium*. A similar trend was recorded by Khan *et al.* (2006).

Table (15). Effect of biofertilizers applied as foliar spray (FS) and/or soil drench (SD) treatments on phosphate solubilization (clear zone diameter cm).

First season (2003-2004)										
Treatment	First cut					Second cut				
Fertilizer / Methods	control	*Azoto.*	*Bacil.*	*Azoto+ Bacil.*	mean	control	*Azoto.*	*Bacil.*	*Azoto+ Bacil.*	mean
FS	0.00	0.11	0.21	0.28	0.15	0.11	0.12	0.35	0.40	0.25
SD	0.10	0.11	0.40	0.30	0.23	0.00	0.13	0.65	0.50	0.32
FS +SD	0.11	0.12	0.52	0.62	0.34	0.12	0.14	0.70	0.80	0.44
Mean	0.07	0.11	0.38	0.40	0.24	0.08	0.13	0.57	0.57	0.34
L.S.D. P≤ 0.05	Methods 0.026 Fertilizer 0.029 Methods *fertilizer 0.027					Methods 0.047 Fertilizer 0.060 Methods *fertilizer 0.080				
Second season (2003-2004)										
Treatment	First cut					Second cut				
Fertilizer / Methods	control	*Azoto.*	*Bacil.*	*Azoto+ Bacil.*	mean	control	*Azoto.*	*Bacil.*	*Azoto+ Bacil.*	mean
FS	0.10	0.12	0.30	0.32	0.21	0.11	0.12	0.39	0.51	0.28
SD	0.11	0.13	0.42	0.48	0.29	0.12	0.14	0.60	0.63	0.37
FS +SD	0.10	0.13	0.60	0.69	0.38	0.13	0.15	0.75	0.87	0.48
Mean	0.10	0.13	0.44	0.50	0.29	0.12	0.14	0.58	0.67	0.38
L.S.D. P≤ 0.05	Methods 0.030 Fertilizer 0.040 Methods *fertilizer 0.042					Methods 0.020 Fertilizer 0.030 Methods *fertilizer 0.180				

Azoto.= Azotobacter chroococcum , Bacil=Bacillus megatherium

e - Soil nitrogen

Data presented in table (16) show the results of soil total nitrogen in all treatments. The data indicated that inoculation process increased the total nitrogen, the slight increase under phosphate dissolving bacteria inoculation may be due to the release of phosphorus which stimulate N_2 fixation by native microorganisms. Inoculation with *A. chroococcum* caused the highest N_2 fixation compared with phosphate dissolving bacteria. Thus, *A. chroococcum* enriched the soil by nitrogen fixation which increased soil fertility. In the present investigation, a mixed inoculation of the *T. vulgaris* plant with *A. chroococcum* + *B. megatherium* (foliar + soil application) enhanced the growth of *Thymus* and increase the soil fertility as affected by

the soil nitrogen. This result is compatible with the finding of Boddy and Dobereiner (1984).

Table (16) Effect of biofertilizers applied as foliar spray (FS) and/or soil drench (SD) treatments on soil nitrogen (ppm).

First season (2003-2004)										
Treatment	First cut					Second cut				
Fertilizer / Methods	control	*Azoto.*	*Bacil.*	*Azoto+ Bacil.*	mean	control	*Azoto.*	*Bacil.*	*Azoto+ Bacil.*	mean
FS	181	231	186	186	211	189	250	190	256	221
SD	185	259	192	192	227	190	267	201	284	235
FS +SD	189	261	209	209	238	194	273	214	319	250
Mean	185	250	195	271		191	263	201.7	286	
L.S.D. P≤ 0.05	Methods 0.94 Fertilizer 1.09 Methods *fertilizer 1.25					Methods 0.80 Fertilizer 0.97 Methods *fertilizer 1.01				
Second season (year)										
Treatment	First cut					Second cut				
Fertilizer / Methods	control	*Azoto.*	*Bacil.*	*Azoto+ Bacil.*	mean	control	*Azoto.*	*Bacil.*	*Azoto+ Bacil.*	mean
FS	190	248	204	252	223	206	257	213	262	234
SD	209	261	210	289	242	212	271	225	305	253
FS +SD	210	268	218	314	252	227	280	236	336	269
Mean	203	259	210	285		214	269	224	301	
L.S.D. P≤ 0.05	Methods 0.84 Fertilizer 0.97 Methods *fertilizer 1.19					Methods 1.58 Fertilizer 1.82 Methods *fertilizer 3.5				

Azoto.= Azotobacter chroococcum/ Bacil=Bacillus megatherium

Antimicrobial Activity of *Thymus vulgaris* Essential Oil Against Some Common Pathogenic Microbes

Antimicrobial activity of *T. vulgaris* essential oil (extracted from mixed biofertilization treatments with foliar spray and soil drench treatments -second cut of second season) against some human and plant pathogenic microbes was detected and represented in table (17) and figure (1). *Aspergillus albus* was more sensitive than *Salmonella typhi* > *Candida albicans* > *Escherichia coli* > *Staphylococcus aureus* > *Rhizoctonia solani* > *Bacillus subtilis* > *Fusarium oxysporum.* The application of biofertilizers increases the antagonistic activity of *T. vulgaris* essential oil against some pathogenic microbes. This result is compatible with the findings of Siddiqui *et al.* (1996), Abd El-Gawad (2003) and Nzeako *et al.* (2006).

Table (17). Antimicrobial activity of *Thymus vulgaris* essential oil against some common pathogenic microbes.

Pathogenic microorganisms	Inhibition zone diameter (cm)
Escherichia coli	5.6
Salmonella typhi	6.0
Staphylococcus aureus	5.5
Bacillus subtilis	4.2
Fusarium oxysporum	3.9
Rhizoctonia solani	4.5
Aspergillus albus	6.1
Candida albicans	5.9

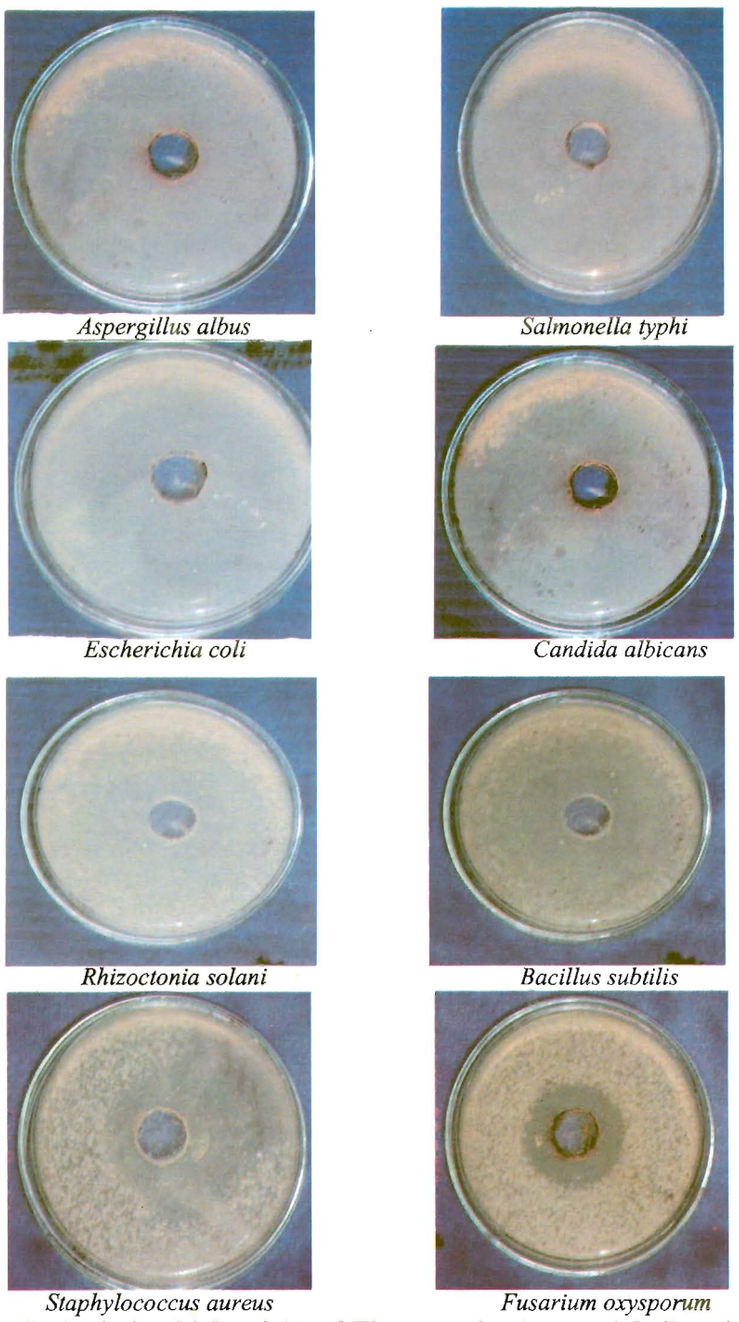

Fig. (1). Antimicrobial activity of *Thymus vulgaris* essential oil against some common pathogenic microbes.

CONCLUSION

From the above mentioned results one can conclude that the use of biofertilization for agriculture in sandy soils under a drip irrigation system gave enhancement effects on the plant growth, yield and essential oil yield also, improved soil characters and increased its fertility.

Application of a mixture of *A. chroococcum* + *B. megatherium* resulted in the highest oil yield productivity compared with individual treatments. A soil and foliar application is preferable to soil or foliar applications only. The most powerful antimicrobial activity against some pathogenic microorganisms appeared in essential oil of mixed biofertilization treatment with foliar spray and soil drench of second cut of second season.

REFERENCES

Abd El-Ghany, B.F.; K.W. Khalil; M. M.El Sersawy and S.Y. Awadalla (1997). Improvement of Wadi Sudr properties using modern bio-organic techniques and their effects on desertification combat and barley production. *Desert Inst. Bull., Egypt.*, 47(1): 69-100.

Abd El-Gawad, A.M. (2003). Biological control of some tomato diseases caused by *Fusarium spp.* and *Alternaria spp. Ph.D. Thesis*, Fac. Sci., Cairo Univ., Giza, Egypt.

Abd El-Gawad, A.M. (1999). Effect of some soil microorganisms on the fertility of Egyptian desert soil. *M.Sc. Thesis,* Fac. Sci., Ain Shams Univ., Cairo, Egypt.

Abd El-Hafez, A.M. (1966). Some studies on acid producing microorganisms in soil and rhizosphere with special reference to phosphate dissolvers. *Ph.D. Thesis*, Fac. Agric., Ain Shams Univ., Cairo, Egypt.

Abd El-Malek, Y. and Y.Z. Ishac (1968). Evaluation of methods used in counting *Azotobacter. J. Appl. Bacteriol.*, 31: 26.

Allen, I.N. (1959). In "*Experiments on soil bacteriology*". Burgess Publishing Co., Minneapolis, Min/ USA.

Attia, M.E. and Hoda H. M. Abdel-Azeem (2004). Effect of biofertilization with some strains of bacteria and chemical fertilization on *Mentha viridis* L. cultivated in Maruit location. *Annals Agric. Sci.*, special issue, 2:431-442.

Badi, N.H.; D. Yazdami ; M.S. Ali and F. Nazari (2004). Effects of spacing and harvesting time on herbage yield and quality/quantity of oil in thyme, *Thymus vulgaris*. *Industrial Crops and Products*, 19(3): 231- 236.

Bergey's Manual of Systematic Bacteriology (1984). Gram positive *Bacillus*. Vol. 1, Section 4, cited from Krieg N.R. and J.G. Holt (eds.), p. 220-229,*Williams and Wilkins, Baltimore, USA*.

Blanco, E.; R. Moralis and R. Pellin (1998). Harvesting and trade of *Thymus* in Spain. In : Traffic Europe (eds.), Medicinal plant trade in Europe. Conservation and supply, p. 50-54. *Proceedings of 1st International Symposium on the Conservation of Medicinal Plants in Trade in Europe*, TRAFFIC- Europe, Brussels/B.

Boddy, R.M. and J. Dobereiner (1984). In "*Nitrogen fixation associated with grasses and cereals*". Current development in biological nitrogen fixation" (edited by Subba Rao, L.N.S.), p. 277-313, Edward Arnold Publishers Ltd., London, UK.

Brown, E.M.; S.K. Burlingham and R.M. Jackson (1964). Studies on *Azotobacter* species in soil. III. Effects of artificial inoculation on crop yields. *Plant and Soil* 20 (2): 194-214.

Bunt, J.S. and A.D. Rovira (1955). Microbiological studies of some subantarctic soils. *J. Soil Sci.*, 6: 119 – 128.

Cherry, J.H. (1973). In "*Molecular biology of plants, a text manual.*" Columbia Univ. Press, New York.

El-Shazly, M.M. (2003). Studies on some factors affecting productivity of growth regulators by *Azotobacter* in desert soil. *M.Sc. Thesis*, Fac. Sci., Al-Azhar Univ., Cairo, Egypt.

Holla, M. and S. Vaverkova (1993). The content and composition of volatile oil from *Salvia officinalis* L. in the dependence on the locality of growing. *Biologia-Bratislava*, 48(6): 619-621.

Khan, S.M.; A. Zaidi and P.A. Wani (2006). Role of phosphate-solubilizing microorganisms in sustainable agriculture. *INRA, EDP Sciences, Agron. Sustain.* Dev. 27: 29-43

Maruzzella, J.C. and J. Balter (1959). The action of essential oils on phytopathogenic fungi. *Plant Disease Reporter*, 43: 1143-1152.

Nzeako B. C.; Zahra S.N. Al-kharousi and Zahra Al-Mahrooqui (2006) Antimicrobial Activities of Clove and Thyme Extracts. *Sultan Qaboos Univ. Med. J.*, 6(1): 74-84.

Revillas, J.J.; B. Rodelas; C. Pozo; M.V. Martinez-Toledo and J.G. Lopez (2005). Production of amino acids by *Azotobacter vinelandii* and *Azotobacter chroococcum* with phenolic compounds as sole carbon source under diazotrophic and adiazotrophic conditions *J. Appl. Microbiology*, 4: 421-425.

Reynders, L. and K. Vlassak (1982). Use of *Azospirillum brasilense* as biofertilizer in intensive wheat cropping. *Plant and Soil*, 66: 217-237.

Shalaby, A.S. and A.M. Razin (1992). Dense cultivation and fertilization for higher yield of thyme (*Thymus vulgaris* L.). *J. Agronomy and Crop Science*, 168: 243-248.

Siddiqui, S.A.; B.C. Dubey; N.P. Shukla; A. Dubey and R.C. Rajak (1996). Interaction between *Azotobacter chroococcum* and rhizosphere microflora of wheat plant. *Advances in Agricultural Research in India*, 6: 64-69.

Snedecor, G.W. and W.G. Cochran (1982). In "*Statistical Methods*". The Iowa State Univ. Press, Ames, Iowa,USA, 507 pp.

Subba Rao, N.S. (1988). In "*Biofertilizers in Agriculture*". Oxford and THB Publ. Co. Ltd., New Delhi, Bombay and Calcutta, p. 134- 141.

Waksman, S.A. and H.A. Lechevalier (1962). In "*The Actinomycetes, Vol. III: Antibiotics of Actinomycetes*". Williams and Wilkins Company, pp. 430, Baltimore/USA.

Youssef, A.A.; A.E. Edris and A.M. Gomaa (2004). A comparative study between some plant growth regulators and certain growth hormones producing microorganisms on growth and essential oil composition of *Salvia officinalis* L. plants. *Ann. Agric. Sci.*, 49(1): 299-311.

Received: 08/10/2007
Accepted: 04/12/2007

استجابة نبات الزعتر النامي فى الأراضي الرملية للتسميد الحيوي تحت نظام الري بالتنقيط

عمرو محمود عبد الجواد وليد محمد عبد العظيم*
قسم خصوبة وميكروبيولوجيا الاراضى – مركز بحوث الصحراء – المطرية – القاهرة – مصر
*قسم النباتات الطبية والعطرية – مركز بحوث الصحراء – المطرية – القاهرة – مصر

أجريت تجربتين حقليتين بمحطة بحوث المغارة التابعة لمركز بحوث الصحراء خلال موسمي ٢٠٠٣-٢٠٠٤ و ٢٠٠٤-٢٠٠٥ لدراسة تأثير التسميد الحيوي باستخدام سلالات الازوتوباكتر كروكوكم (*Azotobacter chroococcum*) كبكتريا مثبتة للنيتروجين وسلالة باسيلس ميجاثيريم *Bacillus megatherium*) كبكتريا ميسرة للفوسفات وقد تم إضافة اللقاحات البكتيرية بثلاث طرق. اضافة للتربة- رش خضرى- اضافه للتربة مع الرش الخضرى (10^8 cfu /ml) على النمو والمحصول ونسبة الزيت الطيار لنبات الزعتر النامي فى الأراضي الرملية وباستخدام نظام الري بالتنقيط.

وقد أظهرت النتائج أن المعاملة باستخدام المخلوط البكتيري عن طريق الاضافة للتربة مع الرش الخضرى تفوقا فى محصول الزيت للفدان والقياسات الخضرية وكذلك التقديرات الميكروبية خلال الموسمين الزراعيين. بينما أظهرت البكتريا المذيبة للفوسفات باسيلس ميجاثيريم (*Bacillus megatherium*) كفاءة اعلى فى تيسير الفوسفات عن بكتريا الازوتوباكتر كروكوكم (*Azotobacter chroococcum*).

وقد أدى استخدام التسميد الحيوى إلى زيادة الكفاءة التضادية للزيت الطيار لنبات الزعتر ضد بعض الميكروبات الممرضة.