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EFFICIENCY OF BIOFERTILIZERS, ORGANIC AND INORGANIC AMENDMENTS APPLICATION ON GROWTH AND ESSENTIAL OIL OF MARJORAM (*Majorana hortensis* L.) PLANTS GROWN IN SANDY AND CALCAREOUS SOILS

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ABSTRACT: A greenhouse pot experiment was set up in order to study the response of sweet marjoram plants to inoculation with tri-mixture of biofertilizers (*Azotobacter* + *Azospirillum* + phosphate dissolving bacteria (PDB)) in the presence of organic and inorganic fertilizers under cultivation in sandy and calcareous soils as representatives of Egyptian desert soils.

Results have shown that marjoram as a medicinal plant normally growing on sandy and calcareous soils in Egyptian desert significantly responded to biofertilization which positively affected plants' growth characters and the essential oil content and it's potency against Gram negative G (-) and G (+) bacteria, fungi and yeast. The best treatment was that including the application of *Azotobacter* + *Azospirillum* + phosphate dissolving bacteria in the presence of K + ¼ N + rock phosphate + sheep dung manure.

Therefore, it could be possible to partially replace biofertilizers instead of inorganic nitrogen and phosphorus fertilizers to reduce the costs of mineral fertilization of both nutrient elements, and avoid the hazard of environmental pollution especially that of nitrate nitrogen.

Key words: Biofertilization, *Azotobacter*, *Azospirillum*, phosphate dissolving bacteria, marjoram.

INTRODUCTION

Any plant or any part of it, that is used as medicine, is called medicinal plant (Lawless, 1992). These plants are used for therapeutic purposes in several ways. Several plants are used either alone or in combination in the traditional systems of medicine. Cultivation and production of these plants has promised future in Egypt, especially in the new reclaimed lands and desert soils.

Sweet marjoram (*Majorana hortensis* L, Family: Labiateae) is considered to be one of the important medicinal plants. It is used in cold disease, stomach and sinews, taken inwardly or outwardly, helped in healing diseases of the chest, obstructions of the liver and spleen. It is also very helpful for muscular and rheumatic pains, sprains, stiff joints, bruises… etc (Lawless, 1992). Also, the antimicrobial action of this plant (the oil, dried whole plants and aqueous extracts derived from the leaves) has only recently received attention in the literature where studies of Deans and Suoboda, (1990); Paster *et al.* (1990); Yavada and Saini, (1991); Ramadan *et al.* (1994), Omer *et al.* (1997); Oberg *et al.* (2000) and Kawabata *et al.* (2003) have been reported and show that the essential oil of sweet marjoram exhibited a significant activity against some plant and animal pathogens, food spoilage organisms and mycotoxins producing molds.

Scientists of the Soil and Microbiology and related disciplines focused their work on the effect of inorganic and/or organic manuring, in addition to biofertilization on growth and yields of vegetables and field crops, but little work has been done, in Egypt, to determine the effect of these forms of fertilization on the growth and productivity of medicinal plants, especially sweet marjoram plants. From these work, in the last two decades, El- Sawy *et al.* (1998) on *Ammi visnaga*; Saleh *et al.* (1998) on *Datura stramonium*; El-Khyat and Zaghloul, (1999) on *Carum carvi*; Nofal *et al.* (2001) on *Ammi visnaga*; Abou-Aly and Gomaa, (2002) on *Coriadrum sativum*; Abou El- Ala, (2002) on *Artemisia cinea*; and Afify, (2002) on *Foeniculum vulgare*.

On the other hand, the extreme condition prevailing our desert soils such as sandy soil of Wadi El- Natrun region and calcareous soil of El- Khatatba region necessitate the introduction of important microorganisms especially those of N_2-fixation and phosphate dissolving bacteria together with organic manuring to increase the productivity of the growing medicinal plants.

Thus, the present work aimed to study the effect of inoculation with biofertilizers (asymbiotic and phosphate dissolving bacteria) in the presence or absence of different forms and doses of organic and mineral fertilization on sweet marjoram plant productivity, oil content, essential oil percentage and oil composition in two different soils (calcareous and sandy soils) in order to improve the growth of this medicinal plant and to maximize its content of medicinal substances. Also, the antimicrobial activity of the essential oil, obtained from the best treatment under this study, was estimated against some tested micro-organisms.

MATERIALS AND METHODS

A greenhouse pot experiment was set up at Soil Fertility and Microbiology Department, Desert Research Center, Ministry of Agriculture and Land Reclamation, Cairo, Egypt, in order to study the response of sweet marjoram plants to bio-organic and inorganic fertilizers under cultivation in calcareous and sandy soils.

Soil Used

Two soils, representing two different types of soils, were obtained from El-Khatatba and Wadi El- Natrun areas and used for cultivation of the tested plant. Before cultivation, soil samples were taken from surface area (0-20 cm) of several spots at each location, air-dried, ground to pass a 2-mm sieve and thoroughly mixed together to give one composite sample. Physico-chemical properties of these soils were determined according to Page *et al.* (1982) as given in Table 1.

Organic Materials Used

Sheep dung manure "SD" and rice straw "RS" were brought from the Mariyut Research Station. These materials were air-dried, ground and milled then sieved (2 mm sieve). They were analyzed at the Soil Laboratory of Desert Research Center Ministry of Agriculture and Land Reclamation, due to the methods recorded in Page *et al.* (1982), Table 2. These materials were added at the rate of 2% of the soil weight before cultivation.

Table 1: Physico-chemical properties of the two tested soils

Parameters	El- Khatatba calcareous soil	Wadi El- Natrun sandy soil
Particle analysis		
Sand	73.90	94.53
Silt	12.49	3.04
Clay	13.61	2.43
Textural class	Sandy loam	Sand
pH	7.50	7.25
E.C. dS/m	1.81	2.13
$CaCO_3$%	30.21	1.06
Organic carbon%	0.10	0.12
Total nitrogen%	0.0204	0.0262
C/N ratio	4.90	4.40
Organic matter%	0.17	0.20
Soluble salts meq/L		
Ca^{++}	8.20	3.80
Mg^{++}	1.80	1.30
Na^{+}	10.50	14.90
K^{+}	0.60	0.90
HCO_3^{-}	1.50	2.30
Cl^{-}	11.20	13.20
SO_4^{--}	8.40	5.40

Table 2: Chemical analysis of organic materials applied to the two studied soils

Characteristics	Sheep dung	Rice straw
Organic carbon %	21.41	38.50
Total nitrogen %	2.12	0.50
C/N ratio	10.10	77.00
Organic matter %	36.82	66.22

Inorganic Fertilizers Used

Ammonium sulphate (21.5% N), calcium super phosphate (15.5% P_2O_5) and potassium sulphate (48% K_2O) were applied according to the recommendation of Abou Zaid (1992) at the rate of 150, 100 and 50 kg/fed., respectively. Nitrogen and potassium fertilizers were added in three split application. The first was 21 days after planting and the other two were applied immediately after the first and second cuts. In case of phosphatic fertilization, it was applied in three split doses. The first one was applied once just before cultivation and the other two doses after the first and second cuts. Also, rock phosphate "RP" was added at the rate of 30 Kg/fed., and it was applied prior to planting.

Seedlings Used

Healthy seedlings of sweet marjoram (*Majorana hortensis* L.) were provided by the Medicinal and Aromatic Plants Department of the DRC, Minsitry of Agriculture and Land Reclamation, Cairo Egypt. They were planted in pots and fertilized with mineral, organic and/or biofertilizers due to the treatments.

Preparation of Inocula and Cultivation

Two efficient isolates of N_2-fixers (*Azotobacter* and *Azospirillum*) and one isolate of phosphate dissolving bacteria were isolated and selected from the soil of the rhizosphere regions of some medicinal and aromatic plants, which commonly grown naturally in Wadi Hagoul region.

Two heavy cell suspensions of efficient isolates of *Azotobacter* and *Azospirillum* were obtained by growing them separately on modified Ashby's medium (Abd El- Malek and Ishac, 1968), and on semi-solid malate medium (Döbereiner, 1978), respectively for 7 days at 28 ± 2°C. Also, heavy cell suspension of phosphate dissolving bacteria was prepared by growing on modified Bunt and Rovira medium (Abd El- Hafez, 1966) for 4 days at 28 ± 2°C.

Suspensions of these isolates containing about 10^8 cell/ml were used as standard inocula. Roots of the seedlings of sweet marjoram were washed by water and then immersed into inoculum for 10 minutes and planted immediately in the pots containing either of the two different soils.

Layout of Pot Experiment and Treatments

In this experiment, pottery pots of 30cm diameter were filled with 7 kg of each soil, and 108 pots were used representing the combination of 9 treatments, 2 soil textures (sandy and calcareous soils), 3 cuts, and 6 replicates. These pots were arranged inside the greenhouse in randomized complete blocks design. The experiment includes the following treatments:

1. Un-inoculated seedlings + full dose of N, P and K.
2. Un-inoculated seedlings + full dose of N, P and K+2% sheep dung manure "SD".
3. Un-inoculated seedlings + full dose of N,P and K+2% rice straw "RS".
4. Inoculation with PDB + full dose of N, K and rock phosphate.
5. Inoculation with *Azotobacter* + *Azospirillum*+full dose of P and K.
6. Inoculation with *Azotobacter* + *Azospirillum*+full dose of P and K+quarter-dose of N.
7. Inoculation with *Azotobacter* + *Azospirillum*+full dose of P and K+half-dose of N.
8. Inoculation with *Azotobacter* + *Azospirillum*+PDB+full dose of K+quarter-dose of N+RP+SD.
9. Inoculation with *Azotobacter* + *Azospirillum*+PDB+full dose of K+quarter - dose of N+RP+RS.

The pots were irrigated with tap water to maintain the soil water content at 50% of the W.H.C. in each pot, and throughout the time course of the experiment. The fertilizers were added to each pot in amounts equal to the recommended dose (dissolved in water), as well as due to the treatments under the study.

Plant Parameters Determination

Fresh and dry weight of roots and shoots of plant samples were determined, and recorded as g/plant, after each cut in both of the two soils. Total nitrogen and total phosphorus were determined in plant samples according to modified Kjeldahl method (Chapman and Pratt, 1961), and ascorbic acid method (Watanabe and Olsen, 1965), respectively.

Oil Parameters Determination

The essential oil percentage in plant samples was estimated according to the A.O.A.C. (1990). The oil extracted from plant samples of control and the oil of the best treatment of the second cut, which gave the highest essential oil percentage were analyzed using Gas Liquid Chromatography. GLC/PYE UNI-CAM PRO-GC, to identify and determine the chemical components of the obtained oils. This analysis was done in the Central Laboratory Faculty of Agriculture, Cairo University.

Antimicrobial Activity of Essential Marjoram Oil

Antimicrobial activity of the tested oil sample, which gave the highest essential oil percentage, was estimated against four bacterial species namely *Bacillus subtilis*, *B. megatherium* (as Gram positive), *Escherischiae coli*, and *Pseudomonos fluorescence* (as Gram negative), one mold (*Fusarium oxysporum*) and one yeast (*Saccharomyces cerevisiae)* using the agar-diffusion technique, (filter paper disc 6 mm), due to the method of Maruzzella and Balter, (1959). The tested organisms were obtained from Microbiology Unit, Fertility and Soil Microbiology Department, Desert Research Center, Cairo Egypt.

Nutrient agar, Czapek's Dox and malt extract agar media were prepared as described by Jacobs and Gerstein (1960), Oxoid (1982) and Harrigan and McCance (1966), then inoculated and incubated to test the antimicrobial activity of marjoram essential oil against the tested bacteria, molds and yeast, respectively. The inhibition zones of the microbial growth were measured (mm).

All samples and determinations were carried out in triplicates, and the data were analyzed according to Statistical Analysis System User's Guide. Analysis of variance was calculated and means were differentiated by using L.S.D (Snedecor and Cochran, 1980).

RESULTS AND DISCUSSION

Shoots Fresh and Dry Weights

Shoots fresh and dry weights of marjoram plants (g/pot) as affected by inoculation with biofertilizers, in addition to organic and/or inorganic fertilizers are given in Table 3. It was generally observed that biofertilizers significantly ($P \leq 0.05$) increased the values of fresh and dry weights of marjoram shoots, as well as do the organic fertilizers.

The highest values were observed when the plants were inoculated with tri-mixture inoculants of Az. + Azosp. + PDB in the presence of K + ¼ N + rock phosphate (RP) + SD (treatment no. 8). This treatment (No. 8) demonstrated that the fresh weight values of marjoram plants growing in sandy soil were 13.9, 33.8 and 38.9g/pot for the first, second and third cuts, respectively.

In calcareous soil, however, the marjoram fresh weight showed 12.0, 33.5 and 46.2g/pot in the three cuts, respectively. The respective weight values of the

Table 3: Shoots fresh and dry weights of marjoram plants (g/pot) in three cuts as affected by applied treatments of fertilization in the two studied soils

Tr.*	First cut						Second cut						Third cut					
	Fresh weight			Dry weight			Fresh weight			Dry weight			Fresh weight			Dry weight		
	Soil I	Soil II	Mean	Soil I	Soil II	Mean	Soil I	Soil II	Mean	Soil I	Soil II	Mean	Soil I	Soil II	Mean	Soil I	Soil II	Mean
1	5.5	5.1	5.3	3.05	2.36	2.70	10.1	10.8	10.4	3.91	3.64	3.77	19.2	14.9	17.1	6.85	6.36	6.61
2	13.5	11.4	12.4	6.86	6.02	6.44	29.5	28.2	28.9	8.24	10.40	9.32	31.2	43.4	37.3	13.26	18.20	15.73
3	6.5	8.1	7.3	3.24	3.50	3.37	17.7	17.9	17.8	5.36	6.39	5.87	22.6	32.7	27.7	8.20	12.76	10.43
4	8.7	8.9	8.8	3.49	4.37	3.93	14.3	19.0	16.6	5.17	6.72	5.94	25.8	37.0	31.4	6.91	13.81	11.71
5	9.2	6.3	7.7	3.74	3.17	3.46	15.7	19.8	17.7	5.53	7.31	6.42	26.4	35.6	31.0	10.31	14.47	12.39
6	9.3	9.1	9.2	4.49	3.62	4.05	16.9	20.7	18.8	5.60	8.14	6.87	27.3	37.1	32.2	10.80	14.79	12.80
7	10.0	9.6	9.8	4:49	3.86	4.18	19.7	21.1	20.4	6.23	9.33	7.78	26.5	41.0	35.2	12.26	16.71	14.48
8	13.9	12.0	13.0	7.26	4.95	6.11	33.8	33.5	33.6	9.99	12.14	11.06	38.9	46.2	42.6	16.44	18.74	17.59
9	6.7	10.0	8.4	3.51	4.15	3.83	22.6	22.7	22.6	7.20	8.30	7.75	32.7	43.7	38.2	12.26	17.48	14.87
Mean	9.3	8.9		4.46	4.00		20.0	21.7		6.36	8.04		28.2	36.9		11.11	14.80	
LSD%																		
Soils			n.s.			0.24			1.0			0.36			0.5			0.31
Treatments			0.8			0.50			2.1			0.76			1.1			0.65
Interaction			1.1			0.71			n.s.			1.08			1.5			0.92

Tr.* = Treatments. Soil I = Wadi El Natrun soil "sandy" Soil II = El Khatatba soil "sandy loam-calcareous"
1. N,P,K 2. N,P,K+SD 3. N,P,K + RS 4. N,K + PDB + RP 5. P,K + Az + Azosp
6. P,K + ¼ N + Az + Azosp 7. P,K + 1/2N + Az. + Azosp. 8. K + 1/4N + SD + Az + Azosp + PDB + RP
9. K + 1/4N + RS + Az. + Azosp + PDB + RP

control (treatment no. 1) were 5.5, 10.1 and 19.2g/pot for those growing in sandy soil and 5.1, 10.8 and 14.9g/pot for the three cuts, respectively, for the plants growing in the calcareous soil. The values of dry weight of shoots behaved similarly in the same trend shown by fresh weight of marjoram shoots in both soils.

These results were true in case of the three cuts with increasing the values in the second and third cuts. Also, El-Khatatba calcareous soil (soil no. II) had higher values of fresh and dry weights of shoots than those exhibited by Wadi El-Natrun sandy soil (soil no. I), especially in the second and third cuts. This could be deduced to the interaction between the genotype of the growing marjoram plants under the cultivation with the different physico-chemical properties of these soils.

These results confirmed the results obtained by Saleh *et al.* (1998), Nofal *et al.* (2001), Abou El-Ala (2002) and Abou Aly and Gomaa (2002). The latter authors showed that dual inoculation of coriander seeds with biofertilizers (*Az. chroococcum* and *Azosp. brasilense*) gave significant increases in vegetative growth of the adopted plants.

In general, the results concluded that inoculation of marjoram plants with tri-biofertilizers of Az., Azosp. and PDB in the presence of sheep dung, rock phosphate, ¼ N and K under cultivation in sandy and calcareous soils of our desert enhanced the vegetative growth and dry matter of shoots of marjoram plants.

Roots Fresh and Dry Weights

Data in Table 4 show that the weights of fresh and dry roots of marjoram plants growing in sandy and calcareous soils as affected by inoculation with biofertilizers together with the application of inorganic and organic amendments. The general trends achieved for the values of fresh and dry weights of roots are similar to those observed for fresh and dry weights of marjoram shoots. Biofertilizers significantly ($P \leq 0.05$) increased the values if they were applied with inorganic and organic amendments.

The highest values of fresh and dry weights of marjoram roots were obtained when the plants were inoculated with tri-mixture biofertilizers of Az. + Azosp. + PDB in the presence of K + ¼ N + rock phosphate (RP) + SD (treatment no. 8). This treatment (No. 8) demonstrated that fresh weight values of roots being 2.98,

Table 4: Roots fresh and dry weights of marjoram plants (g/pot) in three cuts as affected by the applied treatments of fertilization in the two studies soils

Tr.*	First cut						Second cut						Third cut					
	Fresh weight			Dry weight			Fresh weight			Dry weight			Fresh weight			Dry weight		
	Soil I	Soil II	Mean	Soil I	Soil II	Mean	Soil I	Soil II	Mean	Soil I	Soil II	Mean	Soil I	Soil II	Mean	Soil I	Soil II	Mean
1	2.76	2.18	2.47	0.20	0.27	0.24	4.31	3.65	3.98	1.07	0.90	0.98	6.45	4.24	5.34	2.13	1.34	1.74
2	3.27	3.98	3.63	0.54	0.95	0.75	7.87	7.72	7.79	2.92	2.28	2.60	12.18	12.65	12.42	4.21	4.47	4.34
3	2.90	2.87	2.88	0.28	0.64	0.46	6.85	5.15	6.00	1.62	1.38	1.50	7.41	10.60	9.01	2.50	3.51	3.00
4	2.90	2.99	2.94	0.27	0.86	0.56	5.79	5.39	5.59	1.37	1.44	1.41	6.50	10.80	8.65	2.28	3.62	2.95
5	2.88	2.51	2.69	0.25	0.38	0.31	6.95	5.62	6.29	1.78	1.51	1.64	6.80	10.82	8.81	2.39	3.62	3.00
6	2.98	3.22	3.10	0.38	0.89	0.63	7.05	5.84	6.45	1.88	1.54	1.71	7.82	10.92	9.37	2.62	3.68	3.15
7	2.93	3.62	3.28	0.31	0.91	0.61	7.56	6.17	6.86	2.74	1.66	2.20	8.22	11.61	9.91	2.89	4.02	3.46
8	2.98	4.44	4.21	0.83	1.20	1.02	8.93	10.36	9.65	3.16	3.53	3.34	13.17	13.48	13.32	4.62	4.34	4.73
9	2.77	3.71	3.24	0.20	0.92	0.56	7.31	6.48	6.89	2.49	1.77	2.08	7.66	12.65	10.16	2.55	4.46	3.50
Mean	3.04	3.28		0.36	0.78		6.96	6.26		2.11	1.77		8.47	10.86		2.91	3.73	
LSD%																		
Soils			0.01			0.01			0.07			0.04			0.13			0.07
Treatments			0.03			0.01			0.15			0.08			0.28			0.15
Interaction			0.04			0.01			0.21			0.11			0.39			0.21

Tr.* = Treatments. Soil I = Wadi El Natrun soil Soil II = El Khatatba soil

1. N,P,K 2. N,P,K+SD 3. N,P,K + RS 4. N,K + PDB + RP 5.P,K + Az + Azosp

6. P,K + ¼ N + Az + Azosp 7. P,K + 1/2N + Az. + Azosp. 8. K + 1/4N + SD + Az + Azosp + PDB + RP

9. K + 1/4N + RS + Az. + Azosp + PDB + RP

8.93, and 13.17 g/pot for sandy soil in the first , second and third cuts, respectively. The values in calcareous soil were 4.44, 10.36 and 13.48 g/pot in the same order. While control treatment (treatment no. 1) had the lowest values of fresh weights of roots being 2.76, 4.31 and 6.45 g/pot in the same order for the sandy soil, However, the values in the control treatment in calcareous soil were 2.18, 3.65 and 4.24 g/pot in the same order.

In addition, the values of roots fresh weight of marjoram plants grown on calcareous soil exceeded those of the plants grown on sandy soil in the three cuts with greater values in the second and third cuts. Also, the mean differences in the root fresh weight of marjoram reached from 0 to 28% over those growing under sandy soil. This could be explained on the basis of differences between the two different soils of varying fertility status (Russell, 1989).

These results confirmed the findings obtained by Okon (1984) who assured the benefits of *Azospirillum* to plant roots by the mechanisms related to enhancement of plant roots growth and functioning.

In a general conclusion, biofertilizers effect was again very clear in enhancement of the values of fresh and dry weights of marjoram roots under bio-organic and/or inorganic fertilization.

Total Nitrogen Content in Shoots

Data presented in Table 5 demonstrate that the application of inorganic amendments and/or inoculation with different tri-mixture of biofertilizers namely Az. + Azosp. + PDB increased the total nitrogen in the shoots of marjoram plants compared with the control treatment, which received NPK inorganic fertilizers only. In the aforementioned treatment (No. 8), the nitrogen content values of marjoram shoots grown on sandy soil of Wadi El-Natrun were 180, 338 and 636mg/ plant for the first, second and third cuts, respectively. The respective values for plants grown in the calcareous soil of El-Khatatba were 103, 374 and 783mg/plant. However, the respective values of the control (No. 1), which received NPK inorganic fertilizers only without bio-inoculation, were 39, 86 and 142mg/plant for the plants grown in sandy soil and 33, 102 and 190mg/plant for those grown on calcareous soil in the first, second and third cuts, respectively.

However, values of total nitrogen content in the shoots of marjoram plants grown on calcareous soils were higher than those grown on sandy soil only in the second and third cuts. Mean values in the calcareous soil were 63, 216 and 515mg/plant, while they were 82, 161 and 344mg/plant in the sandy soil in the three cuts, respectively. This is due to the differences in the physico-chemical properties and fertility status of the two soils.

In this concern, Saleh *et al.* (1998), in their work on datura plants (*Datura stramonium*), found that dual inoculation with asymbiotic N_2-fixers (Az. + Azosp.) and VAM in treatments supplemented with full dose of rock phosphate significantly increased dry matter, nitrogen content and alkaloids in datura plants. Recently, Abou-Aly and Gomaa (2002) showed that dual inoculation of coriander seeds (*Coriandrum sativum*) with mixed microbes of non-symbiotic N_2-fixers (*Azotobacter chroococcum* and *Azospirillum brasilense*) and phosphate solubilizers gave significant increases in vegetative growth, nitrogen content and essential oil content of treated plants.

Generally, the results concluded that inoculation with biofertilizers (Az. + Azosp.+ PDB) in the presence of inorganic plus organic amendments increased total nitrogen in the shoots of marjoram plants.

Total Nitrogen Content in Soil

Data in Table 6 show the soil nitrogen content as affected by cultivating marjoram plants in sandy and calcareous soil treated with organic and inorganic and/or inoculated with tri-isolates of non-symbiotic N_2-fixers and phosphate dissolving bacteria. Generally, soil nitrogen content increased in both soils as a result of treatment with biofertilizers and/or organic manure as compared with the control treatment. However, the magnitude of increase varied according to soil type as well as to the applied treatment.

Again, treatment no. 8, which received bio-organic fertilizer + K + ¼ N + rock phosphate + SD, gave the highest values of soil nitrogen in both sandy and calcareous soils with higher values in the latter soil. Soil nitrogen content values were 910, 1330 and 2365mg/kg dry soil in the sandy soil compared with 1330, 1540 and 2540mg/kg dry soil in the calcareous soil in the three cuts, respectively.

These results are in agreement with those reported by Ishac *et al.* (1986), Fayez (1990), El-Demerdash *et al.* (1993) and Hegazi *et al.* (1993). They attributed the increases in soil

Table 5: Total nitrogen content (mg/plant) of marjoram plants in three cuts as affected by the applied treatments of fertilization in the two studied soils

Treatments	First cut			Second cut			Third cut		
	Soil I	Soil II	Mean	Soil I	Soil II	Mean	Soil I	Soil II	Mean
1	39	33	36	86	102	94	142	190	166
2	102	84	93	188	300	244	355	706	531
3	45	42	44	96	190	143	187	441	314
4	73	61	67	133	167	150	296	453	375
5	63	50	57	116	146	131	307	453	380
6	94	72	83	150	230	190	396	574	485
7	80	65	73	117	188	153	316	414	365
8	180	103	142	338	374	256	636	783	710
9	59	53	56	222	247	235	463	617	540
Mean	82	63		161	216		344	515	
LSD (5%)									
Soils			1			2			3
Treatments			1			3			7
Interaction			2			5			10

Abbreviations: as those recorded in Table (3)

Table 6: Soil total nitrogen content (mg/kg dry soil) under marjoram plants in three cuts as affected by the applied treatments of fertilization in the two studied soils

Treatments	First cut			Second cut			Third cut		
	Soil I	Soil II	Mean	Soil I	Soil II	Mean	Soil I	Soil II	Mean
1	315	560	438	578	840	709	1000	1120	1060
2	420	750	585	1110	1260	1185	1330	1400	1365
3	355	700	528	840	1120	980	1160	1220	1190
4	630	420	525	630	750	690	1120	1350	1235
5	560	420	490	633	1125	879	1126	1260	1193
6	700	805	753	770	1190	980	1400	1500	1450
7	595	770	683	1190	1405	1298	1560	1540	1550
8	910	1330	1120	1330	1540	1435	2365	2540	2453
9	420	1050	735	700	1330	1015	1195	1470	1333
Mean	545	756		865	1173		1362	1489	
LSD (5%)									
Soils			26			41			57
Treatments			39			61			86
Interaction			59			92			128

Abbreviations: as those recorded in Table (3) nitrogen content to the inoculation with *Azotobacter* and *Azospirillum* owing to the great amounts of asymbiotic N_2-fixation in the soil solution, which could be the reason of the increases obtained in soil nitrogen content and plant growth.

These results concluded that soil nitrogen content increased in both sandy and calcareous soils as a result of inoculation with biofetilizers and/or organic and inorganic amendments. The magnitude of increase varied according to soil type as well as to the applied treatment.

Total Phosphorus Content in Shoots

Values of total phosphorus content in marjoram plants as affected by inoculation with biofertilizers and/or organic amendment application are shown in Table 7. Phosphorus contents in marjoram plants grown on calcareous soil of El-Khatatba were higher than those observed in plants grown on the sandy soil of Wadi El-Natrun, especially in the second and third cuts, being in the mean values of 9.0, 30.9 and 46.3mg/plant for calcareous soil and 9.3, 22.6 and 34.5mg/plant for sandy soil in the first, second and third cuts, respectively.

In general, treatment no. 8, in which the soil received potassium (K) + ¼ N + rock phosphate + sheep dung (SD) as organic manure and inoculated with tri-mixture of Az. + Azosp. + PDB, gave the highest values of plant phosphorus. These results emphasize the use of biofertilizers to replace the inorganic nitrogen and phosphorus to combat the hazard of environmental pollution and to realize economic importance in the production cost of such medicinal plants (Ishac *et al.*, 1993; Saleh *et al.*, 1998; Abou-Aly and Gomaa, 2002 and Migahed *et al.*, 2004).

These results confirmed the findings obtained by Abou-Aly and Gomaa (2002). They found that inoculation of coriander plants (*Coriandrum sativum*) with *Az. chroococcum* or *Azospirillum brasilense*, combined with *Glomus mosseae* gave significant increases in N, P and K contents in the plants. In the same line, Abd El-Ghany (1994) reported that inoculating fodder beat plants with phosphate dissolving bacteria (PDB) namely *B. megatherium* in the presence of rock phosphate facilitated the solubilization of phosphate in the soil by producing organic acids which lower the pH and bring about the dissolution of bound form of phosphate. Recently, Brock (2003) added that some of hydroxy acids may chelate with calcium and iron resulting in effective solubilization and utilization of phosphate.

In general conclusion, these results emphasize the use of biofertilizers and phosphate dissolving bacteria (PDB) to increase the total phospharus content in marjoram plants.

Essential Oil Content (%)

The essential oil content (%) in marjoram plants in the three cuts as affected by the applied treatments of fertilization in the two studied soils is given in table (8). It could be observed that there were significant increases in the values of essential oil content (%) due to the application different bio-fertilization treatments as compared with the control, which received N, P and K only without biofertilization. These results are consistent with those obtained by Neelima and Janardhanan (1996). They reported that dual inoculation of VAM + *Azospirillum brasilense* significantly increased the growth, yield and oil content of palmarosa (*Cybopogon martinii* var. motia) over the un-inoculated control.

Data presented in Table 8 show also that treatment no. 8 in both soils with K + ¼ N + rock phosphate + sheep dung and inoculating the plants with biofertilizers (Az.+ Azosp. + PDB) gave the highest oil percentage in marjoram plants as compared with the control. The percentages of oil content in marjoram plants in treatment no. 8 were 2.30, 2.33 and 2.25% in plants grown on sandy soil and were 1.77, 1.81 and 1.74% in those grown on calcareous soil in the first, second and third cuts, respectively. The respective values for control treatment being 0.63, 0.66 and 0.61% in sandy soil and 1.11, 1.15 and 1.06% in calcareous soil in the same order.

These results are supported by the findings of Ibrahim (2000) who found that inoculation of sweet fennel plants with *Azotobacter* and *Azospirillum* in the presence of N, P and K gave the tallest plants, more umbles per plant and higher seed and oil yields. Also, Abou-Aly and Gomaa (2002) obtained similar results with coriander (*Coriandrum sativum*) concerning the increase in its oil content due to biofertilizer application using *Az. chroococcum* or *Azosp. brasilense* combined with *B. megatherium* var. phosphaticum.

It could also be seen that the oil percentages in marjoram plants grown on sandy soil of Wadi El-

Table 7: Phosphorus content (mg/plant) of marjoram plants in three cuts as affected by the applied treatments of fertilization in the two studied soils

Treatments	First cut			Second cut			Third cut		
	Soil I	Soil II	Mean	Soil I	Soil II	Mean	Soil I	Soil II	Mean
1	4.6	3.6	4.1	8.5	8.7	8.6	13.8	12.6	13.2
2	11.1	11.3	11.2	24.1	31.9	28.0	35.0	42.6	38.9
3	4.6	6.1	5.4	14.2	18.6	16.4	19.4	29.0	24.2
4	7.7	10.5	9.1	20.4	26.8	23.6	35.7	43.8	39.8
5	7.6	6.9	7.3	21.1	27.5	24.3	37.7	44.4	41.1
6	9.6	7.9	8.8	19.8	30.4	25.1	32.1	44.5	38.3
7	9.5	8.6	9.1	21.4	35.3	28.4	35.7	50.0	42.9
8	20.0	14.5	17.3	47.0	59.5	53.3	60.7	78.3	69.5
9	8.6	11.4	10.0	27.1	39.3	33.2	40.3	71.7	56.0
Mean	9.3	9.0		22.6	30.9		34.5	46.3	
LSD (5%)									
Soils			0.1			0.2			0.3
Treatments			0.2			0.5			0.7
Interaction			0.2			0.7			1.0

Abbreviations: as those recorded in Table (3)

Table 8: Essential oil content (%) of marjoram plants in three cuts as affected by the applied treatments of fertilization in the two studied soils

Treatments	First cut			Second cut			Third cut		
	Soil I	Soil II	Mean	Soil I	Soil II	Mean	Soil I	Soil II	Mean
1	0.63	1.11	0.87	0.66	1.15	0.91	0.61	1.06	0.84
2	1.67	1.39	1.53	1.69	1.42	1.56	0.61	1.38	1.50
3	1.36	1.21	1.29	1.40	1.25	1.33	1.15	1.20	1.18
4	1.39	1.37	1.38	1.42	1.43	1.43	1.36	1.38	1.37
5	1.28	1.46	1.37	1.35	1.53	1.44	1.29	1.46	1.38
6	1.70	0.94	1.32	1.77	1.17	1.47	1.67	1.06	1.37
7	1.25	1.57	1.41	1.31	1.62	1.47	1.26	1.56	1.41
8	2.30	1.77	2.04	2.33	1.81	2.07	2.25	1.74	2.00
9	0.73	0.52	0.63	0.82	0.60	0.71	0.75	0.54	0.65
Mean	1.37	1.26		1.42	1.33		1.33	1.26	0.65
LSD (5%)									
Soils			0.01			0.01			0.01
Treatments			0.01			0.01			0.01
Interaction			0.02			0.02			0.02

Abbreviations: as those recorded in Table (3)

Natrun, in most treatments, exceeded those plants grown on calcareous soil of El-Khatatba. This could be deduced to the difference in the genotype and nature of the plants behaviour under the conditions of different soil types. Moreover, calcareous soil contains high amount of calcium, which could be detrimental to the oil percentage of marjoram plants. Kozlowski and Novak (1983) reported that marjoram plants receiving no Ca had greater essential oil content and gave a higher yield than plants receiving Ca at 10.0g/pot plus 1.2g N/pot as N-fertilizer containing Ca.

In general conclusion, these results indicate the usefulness of biofertilizers application in place of inorganic nitrogen and phosphorus fertilizers.

Essential Oil Components

Two samples of the essential oil, which was extracted from marjoram plants represented the application of biofertilization, organic and inorganic amendments (treatment no. 8) compared with that extracted from the control plants in each of the studied soils, were subjected to Gas Liquid Chromatograph analysis (GLC) to recognize their profile. Data in Table 9 show the percentages of the essential oil components according to the GLC analysis of samples obtained from plants grown on sandy soil of Wadi El-Natrun as well as from those on calcareous soil of El-Khatatba region.

Table 9: Gas chromatograph analysis of the essential oil of marjoram plants (%)

Components	Sandy soil		Calcareous soil	
	Control	Best treatment	Control	Best treatment
Y-Terpinene	2.04	1.21	0.72	0.99
Linalool	12.67	9.54	6.60	7.50
Borneol	5.26	19.30	27.03	24.96
Ocimene	12.79	19.30	27.03	24.96
Me-Chavicol	8.15	8.56	4.00	6.60
Linalyl acetate	8.15	8.56	4.10	6.60
α-Terpinene	8.15	8.56	4.10	6.60
Carvacrol	0.83	0.91	1.35	0.81
Feranyl acetate	1.65	1.64	1.35	1.23
Eugenol	1.65	1.12	0.48	0.11
Thymol	0.63	1.12	0.48	0.84

Data revealed that the components of the essential oil of marjoram plants grown on both soils are the same in both samples, control and biofertilization plus organic and inorganic amendment treatments. The GLC profile of the essential oil of all plants showed eleven compounds namely γ-terpinene, linalool, borneol, ocimene, Me-chavicol, linalyl acetate, α-terpinene, carvarol, feranyl acetate, eugenol and thymol.

However, there were clear differences in the quantities of the different constituents of the essential oil between the treated plants with biofertilizers and those of the control. The highest concentrations of the different compounds in the profile of essential oil of marjoram plants growing in sandy soil of Wadi El-Natrun, of the control treatment were linalool (12.67%), ocimene (12.79%), Me-Chavicol (8.15%), linalyl acetate (8.15%) and α-terpinene (8.15%). the respective values for plants treated with biofertilizers together with organic and inorganic amendments (treatment 8) were 9.54% for linalool, 19.3% for ocimene, 8.56% for Me-Chavicol, 8.56% for linalyl acetate and 8.56% for α-terpinene. However, borneol and ocimene showed the highest percentages in the treated plants as major oil constituents of marjoram plants essential oil, where they both reached 19.3% as compared with the values obtained from the oil of the control plants, which were 5.26% for borneol and 12.79% for ocimene.

This finding refers to that biofertilization and organic and inorganic fertilizer application to marjoram plants could have a stimulating effect on some components of the essential oil extracted from marjoram plants grown on sandy soil, as borneol and ocimene, while its effect on the other constituents was very slight.

Regarding the essential oil of marjoram plants grown on calcareous soil of El-Khatatba region, the profile showed the same compounds in the control and treated plants like those grown on sandy soil of Wadi El-Natrun. However, the concentrations of different compounds showed higher percentages for borneol and ocimene as compared with the plants grown on sandy soil being 5.26 and 19.3% for borneol and 12.79 and 19.3% for ocimene in control and treated treatments in sandy soil. The respective values in calcareous soil were 27.03 and 24.96% for both borneol and ocimene in control and treated treatments, respectively.

In the same subject, Refaat *et al.* (1990) found that the Egyptian marjoram oil on GC analysis was richer in the main component, terpinen-4-ol (28.9%). Also, Baser *et al.* (1993) reported that the major component in the essential oil of *Origanum marjorana* L. was carvacrol (78.3-97.5%). In addition, Arnold *et al.* (1993) identified thirty-nine components and the main constituents of *Origanum marjorana* were cis-sabinene hydrate, cis-thuyanol-4 (7.4-33.3%) and terpinen-4-ol (16.6-21.6%).

Antimicrobial Action

Table 10 shows the inhibition zones (mm) due to the anti-microbial action of the essential oil extracted from marjoram plants grown in sandy and calcareous soils against G (-) and G (+) bacteria, fungi and yeast. Data show that the oil obtained from plants treated with different amendments significantly showed high values of inhibition zones as compared with the control. In addition, treatment with biofertilizers (Az.+Azosp.+PDB) and organic and inorganic fertilizers (treatment 8) gave the best results concerning the oil potency against *E. coli* (28 and 27mm), *Ps. fluorescence* (37 and 37mm), *B. subtilis* (27 and 26mm), *B. megatherium* (26 and 25mm), fungi (21 and 23mm) and yeast (34 and 39mm) in both sandy and calcareous soils, respectively.

These findings refer to that biofertilization and/or organic and inorganic amendments gave healthy plants containing oil of higher potency as anti-microbial substances. The results are in harmony with those obtained by Migahed *et al.* (2004) who revealed that essential oil obtained from *Apium graveolens* plants gave different antagonistic effects against different tested organisms. The least inhibition zones were observed with un-inoculated control, while the highest inhibition zones were observed with the treatment including tri-mixture inoculation (Az. + Azosp. + PDB) being 26, 24, 22, 20 and 29mm against *E. coli, Candida, B. subtilis, R. solani* and *F. oxisporum*, respectively.

It can be concluded that marjoram plants as medicinal plants normally grown on desert areas significantly responded to

Table 10: Antimicrobial action of marjoram essential oil against bacteria, fungi and yeast calculated as the diameter of inhibition zone (mm) as affected by the applied treatments in the two studied soils

Treatments	Gram negative bacteria *E. coli*			*Ps. Fluorescnece*			Gram positive bacteria *Bacillus subtilis*			*Bacillus megatherium*			Fungi *Fusarium oxisporum*			Yeast *Sacch. cerevisiae*		
	Soil I	Soil II	Mean	Soil I	Soil II	Mean	Soil I	Soil II	Mean	Soil I	Soil II	Mean	Soil I	Soil II	Mean	Soil I	Soil II	Mean
1	18	17	18	19	19	19	15	15	15	17	16	17	16	15	16	22	21	22
2	20	20	20	19	21	20	16	16	16	16	17	17	16	16	16	23	22	23
3	22	21	22	21	25	23	16	18	17	19	18	19	17	18	18	23	25	24
4	22	22	22	25	25	25	18	19	19	21	20	21	18	17	18	22	27	25
5	26	25	26	28	27	28	17	20	19	19	19	19	21	22	22	27	28	28
6	25	21	23	31	29	30	19	19	19	21	21	21	21	21	21	30	36	33
7	25	25	25	33	33	33	22	23	23	22	21	22	22	21	22	30	37	.34
8	28	27	28	37	37	37	27	26	27	26	25	26	21	23	22	34	39	37
9	25	25	25	31	35	33	21	23	22	20	25	23	21	20	21	31	33	32
Mean	23	23		27	28		19	20		20	20		19	19		27	30	
LSD%																		
Soils			1			1			1			1			1			1
Treatments			2			2			2			2			2			3
Interaction			3			4			3			3			3			4

Tr.* = Treatments. Soil I = Wadi El Natrun soil Soil II = El Khatatba soil

1. N,P,K 2. N,P,K+SD 3. N,P,K + RS 4. N,K + PDB + RP 5. P,K + Az + Azosp
6. P,K + ¼ N + Az + Azosp 7. P,K + 1/2N + Az. + Azosp. 8. K + 1/4N + SD + Az + Azosp + PDB + RP
9. K + 1/4N + RS + Az. + Azosp + PDB + RP

bio-fertilization, which positively affected plants growth characters and their essential oil content. The anti-microbial activities of marjoram essential oil against G(-) and G(+) bacteria, as well as, fungi and yeast were also positively affected due to bio-fertilization and/or organic manuring.

Therefore, it could be possible to partially replace biofertilizers instead of inorganic nitrogen and phosphaorus fertilizers to reduce the costs of fertilization, in specific, and avoid the hazard of environmental pollution, in general.

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كفاءة استخدام الأسمدة الحيوية، العضوية والغير عضوية على النمو والزيت الطيار لنباتات البردقوش النامية فى الأراضى الرملية والجيرية

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أجريت تجربة في الصوبة الزراعية لدراسة استجابة نبات البردقوش للتلقيح بخليط من الأسمدة الحيوية المثبتة للنتروجين الجوي (الأزوتوباكتر + الآزوسبيريللم + البكتريا المذيبة للفوسفات) في وجود الأسمدة العضوية والمعدنية والزراعة في تربة رملية وجيرية كمثال للأراضي الصحراوية المصرية. وقد بينت النتائج أن نبات البردقوش كنبات طبيٍ ينمو في الأراضي الرملية والجيرية فإنه يستجيب للتسميد الحيوي والذي يؤثر إيجابياً على صفات النمو وكذلك يؤثر على زيوت النبات الطيارة وأيضاً على البكتريا السالبة والموجبة لجرام بالإضافة إلى أن الفطر والخميرة أيضاً يتأثران إيجابياً وهذا يعزى إلى التسميد الحيوي و/أو التسميد العضوي في المناطق الصحراوية. وكانت أفضل معاملة هى المتضمنة استخدام الأزوتوباكتر + الأزوسبيريللم + البكتيريا المذيبة للفوسفات + البوتاسيوم + ¼ جرعة النيتروجين + صخر الفوسفات + سماد الغنم. ولذلك فإنه من الممكن إحلال التسميد الحيوي إحلالاً جزئياً بدلاً من التسميد المعدني النتروجيني والفسفوري لتقليل تكاليف الأسمدة الكيماوية بالإضافة إلى تجنب أو التخلص من التلوث البيئي.