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EFECT OF MINERAL AND BIO-PHOSPHATE FERTILIZATION ON THE GROWTH, ESSENTIAL OIL PRODUCTIVITY AND CHEMICAL COMPOSITION OF MARJORAM PLANT

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ABSTRACT

Two field experiments were carried out during two summer successive seasons of 2004 and 2005 at the Experimental Station of the Medicinal and Aromatic Plants, Fac. Agric., Mansoura Univ. to evaluate the effect of phosphorus fertilizer levels (0, 15.5, 23.25, and 31 kg P_2O_5 / fed) with or without bio-fertilizers (VA-Mycorrhizae and phosphorein) on vegetative growth, herb yield and essential oil content as well as mineral elements (N, P and K) of marjoram plant.

Data revealed that plants treated with phosphorus recorded considerable increments with regard to growth characters, herb yield, essential oil content, components and mineral elements. The best results in this concern were obtained by using the highest level of phosphorus (31kg P_2O_5 / fed).

Also, obtained data showed that bio-fertilizers (VA-Mycorrhizae and phosphorein) improved the growth characters expressed as plant height, number of branches, plant fresh and dry weight, herb yield, essential oil content and mineral elements compared with control.

The interaction between mineral and bio-phosphate improved plant growth which gave higher herb yield, essential oil content and mineral elements resulted from Mycorrhizae treatment with 23.25 or 31 kg P_2O_5 / fed, compared with other treatments. The highest values resulted from Mycorrhizae with 31 kg P_2O_5 / fed treatment plants.

INTRODUCTION

An increasing interest in the cultivation and production of medicinal and aromatic plants has bean recently noticed in Egypt in order to cover the increasing demands of the local industries as well as export purpose, among these plants marjoram.

M*ajorana hortensis*, L. (Fam. *Lamiaceae*), is indigenous to the Eastern Mediterranean countries and is cultivated commercially in Asia, Southern Europe and some areas in the United States. The plant is perennial, bushy herb approximately grows 60 cm height with small white, sometimes pinkish flowers in tight. The leaves are oval about 2.5 cm long, dusty green, very aromatic when crushed and bloooms June to August (Keville, 1999). Marjoram is a tender plant and is usually grown as an annual. It is rightly named sweet marjoram; the leaves are sweetest when taken just before the plant flowers. It is entirely aromatic in all parts and the aroma is strong. Dried leaves and the oil used as spices in the food industry (Sanecki, 1975).

Oil of marjoram is an essential oil obtained by steam distillation from the dried herb, including terpenes, terpineols, esters and ketones. This oil plays a minor role in perfumery (Panda, 2000).

Dried leaves as well as the essential oil are widely used in many industries and pharmaceutical preparations as stimulating, antiseptic, carminative, digestion and antioxidant (Bellakkadar *et al*., 1988).

Phosphorus is considered the second essential nutrient element in both plants and microorganisms. Characteristically, under phosphorus deficiency shoot growth is much more depressed than photosynthes. The finely tuned homeostasis of phosphorus in the cytosol and chloroplasts is one reason for this and a higher activity of various enzymes of carbohydrate metabolism (Rao *et al*., 1990). Of course, with severe phosphorus deficiency various parameters or photosynthesis are impared (Lauer *et al*., 1989). In spite of the considerable addition of phosphorus to soil, the amount available for plant is usually low. Mohamed and Abd El-Hafez (1982) reported that after fertilization with calcium super phosphate, the level of available phosphorus decreases sharply after a short period from application. They added that this case is widespread in alkaline soils, since the available phosphorus in the added fertilizer is rapidly transformed to tricalcium phosphate, thus, become unavailable to the plant.

With respect to the repromoting effect of P-fertilizer on growth of plants, this may be due to that phosphate regulates enzymatic processes, the phosphorylation of adenosine diphosphate (ADP) to adenosine triphosphate (ATP). Also, phosphate acts as an activator for some enzymes, leading to enhancement of the metabolism process and formation of new cells (Dhillon, 1978). Mousa (1990) reported that invariable produce profitable responses. Phosphorus is necessary for protoplasm formation, and considerable influence by the quantity of plant available nitrogen. Ahmed and Zayed (1994) mentioned that treated plants of fenugreek with 200 kg / fed calcium super phosphate gave the highest values of plant height, number of branches, plant fresh and dry weights The phosphorus alone generally enhanced the growth, yield parameters and volatile oil content of *Nigella sativa,* L. plants (Mohamed *et al.,* 2000).

Balanced nutrition is very important for obtaining vigorous vegetative growth, high production and good quality. Nile valley soils faced numerous deteriorating problems during the last decades, among which is the shifting to the alkaline side, using plant nutrients in unavailable forms. Most farmers are applying intensive and non-rational rates of mineral fertilizer. Most of these fertilizer elements are either fixed in the soil or leached and become insaccessible by plant. Several investigators indicated that soil inoculation with phophorus solubilizing bacteria improved soil fertility and plant productivity by releasing phosphorus element (Hauka *et al*., 1990). Also, El-Sheekh (1997) stated that, under Egyptian soil conditions, using bio-fertilizer phosphate with or instead of mineral phosphate apparently increased the available P concentrations in both soil and plants.

Furthermore, the excessive use of mineral fertilization represents the major cost of crop production and creates pollution of agroecosystem. Kandeel and Sharaf (2003) on marjoram, Eisa (2004) on sage and Heikel (2005) on thyme mentioned that bio-fertilizer (phosphorein) increased vegetative growth, herb yield, volatile oil percentage and chemical components (N, P and K).

Mycorrhizae fungus is one of the most important bio-fertilizers, hence these fungi link plant and soil, transport nutrients to the plant roots and carbon compounds to the soil. Mosse (1981) found that Mycorrhizae fungi may enhance plant growth by improving the supply of nutrients of low mobility in soil by direct and indirect modifications in the rhizosphere. The most distinct growth enhancement effect by VAM occurs by improving supply of mineral nutrients of low mobility in the soil solution, predominantly phosphorus. External hyphae can absorb and translocate phosphorus to the host from soil outside the root depletion zone of non-mycorrhizae root supply. As a rule in VAM plants uptake, the rate of phosphorus per unit root length is 2 - 3 time higher than in non-mycorrhizae plants (Nielsen and Jensen, 1983).

The influence of application of Mycorrhizae on some medicinal and aromatic plants has been studied by many workers. Ezawa & Yoshida (1994) on *Tagetes patula* plants, Kandeel & Sharaf (2003) on *Majorana hortensis* plants, Eisa (2004) on *Salvia officinalis* plants and Mohamed & Saad (2004) on *Achillea milefolium* plants showed that, inoculation with VAM increased growth parameters, herb yield, volatile oil content and chemical composition when compared with control.

The use of symbionts is more economical and much better than the use of chemical fertilizers, which has already raised the pollution of the environment. Thus, attention should be directed in Egypt, to the use of microorganisms as bio-fertilizers to provide nutrients for higher plants without any pollution to the environment.

This study aimed to explore the response of marjoram plants growth, herb yield and essential oil productivity to mineral and / or bio-fertilizer phosphorus treatments as well as their interaction.

MATERIALS AND MOTHEDS

Two field experiments were carried out during two successive summer seasons of 2004 and 2005 at the Farm of Medicinal Plants, Fac. Agric., Mansoura Univ. to study the response of marjoram plants growth, herb yield and essential oil productivity to mineral and / or bio-fertilizer treatments of phosphorus as well as their interaction.

Seeds of marjoram were sown in prepared nursery beds on October 5th in both seasons. The growing seedlings were transplanted after 80 days from sowing at 30 cm apart on the eastern side of row in an irrigated soil. The soil of the experimental location was clay-loam.

Randomized soil samples were obtained from the field to determine the physical and chemical contents according to the standard method described by Wilde *et al*. (1985). Soil properties are presented in Table A.

Table (A) : Physical and chemical properties of the experimental soil in the two seasns 2004 and 2005.

Clay (%)	Silt (%)	Sand (%)	Organic matter (%)	pH	Available nutrients (ppm)					
					N	P	K	Zn	Fe	Mn
40.7	33.2	26.1	2.01	8.18	53.7	12.9	392	1.42	8.35	12.86
40.4	33.6	26.3	2.03	8.21	53.2	12.5	367	1.34	7.79	12.54

Each experiment included twelve treatments representing the interaction between four application rates of mineral phosphorus 0, 15.5, 23.25 and 31 kg / fed as calcium super phosphate (15.5 % P_2O_5) with or without bio-fertilizers (VA-Mycrohizae and phosphorein). The phosphorus fertilizer levels were randomly located in the main plot, whereas, the sub-plots were devoted for bio-fertilizers. The sub-plot area was 4.5 m^2, which consisted of 5 ridges. Each ridge was 1.5 m length containing 5 plants at distance of 30 cm and between ridges was 60 cm. Mineral and bio-phosphate fertilizers were added during the field preparation. The nitrogen and potassium mineral fertilizers were applied as the recommended rates, 200 kg / fed ammonium sulfate (20.5 % N) and 100 kg / fed potassium sulfate (48 % K2O).

In both seasons, the plants were harvested three times yearly by cutting the aerial parts of each plant (10 cm) above the soil surface. The first cut was on May 15^{th} (at commencement of flowering), the second one was done on July 15^{th}, while the third one was done on September 15^{th} (two month intervals). Five plants were randomly chosen from each experimental unit at the three cuts, respectively in both seasons. The vegetative growth parameters [plant height number of branches, plant fresh and dry weight and herb yield] were recorded.

The essential oil percentages were determined from dry leaves using 100 g samples for each cut. The distillation of essential oil and the determination were described in the Egyptian Pharmacopoeia (1984). The oil content was calculated by multiplying oil percentage by weight of dry leaves per plant (ml / plant) and per feddan (Liter / fed). The essential oil obtained from the second cut of the second season was analyzed using Gas Liquid Chromatography Technique (GLC), which carried out at the Central Laboratory, Fac. Agric. Cairo Univ.

The quantitative determination of main components of oil samples were subjected to GLC analysis and calculated following the methods of Guenther and Joseph (1978).

Mineral elements was carried out in the Laboratory of Chemical Dept., Fac. Agric. Mansoura Univ. Leaf samples were dried in an electric oven at 70ºC for 48 hrs then finely ground for chemical determination according to A.O.A.C. (1970). Nitrogen percentage was determined according to micro-kjeldahl method Jackson (1967). Phosphorus percentage was determined colorimetrically according to Murphy and Reily (1962). Potassium percentage was determined using the Atomic Absorption Spectrophotometer (3300) according to Wilde *et al.* (1985).

After all cuts at 2005 season rhizospheric soil was micro-biologically analyzed for the desities of phosphoate-solubilizing bacteria on Allen (1969) medium. VAM fungi spores were collected from soil samples by sieving-decanting method individual spores had to be hand for identification (Trappe 1982).

The split-plot design in a compeletly randomized block with 3 replicates was used in both growing seasons. Obtained data were subjected to the statistical analysis of variance (ANOVA) in split plot design as mentioned by Gomez and Gomez (1984).

RESULTS AND DISCUSSIONS

1- Vegetative growth

Data presented in Tables (1 and 2) indicated that there were significant differences among all treatments. Phosphorus fertilization applied at the highest rate 31 kg P_2O_5 / fed significantly increased stem length and number of branches compared to other treatments at the three cuts in both seasons. The application of phosphorien and Mycorrhizae treatments gave significant differences over the control treatment at the three cuts of the two seasons.

Table (1) : Effect of mineral and bio-phosphate fertilizers and their combination on plant height (cm) of marjoram plant during 2004 and 2005 seasons for three cuts.

Treatments		Plant height (cm)					
		First season			Second season		
P_2O_5 (kg / fed)		1st cut	2nd cut	3rd cut	1st cut	2nd cut	3rd cut
0.0		41.99	44.70	37.91	42.08	44.44	39.29
15.5		45.72	48.63	42.23	46.53	49.06	42.64
23.25		50.81	52.46	46.91	50.99	53.01	46.87
31		53.73	54.08	49.44	53.83	54.85	49.05
LSD at 0.05		3.25	1.54	1.94	3.42	2.45	1.79
Bio-phosphate							
0.0		44.31	47.80	41.88	44.89	47.78	42.04
Phosphorein		48.38	50.00	43.91	48.53	50.46	44.10
VAM		51.50	52.10	46.58	51.66	52.78	47.25
LSD at 0.05		2.15	1.48	1.85	2.21	2.12	1.67
Interaction							
0.0	0.0	37.58	42.58	35.45	36.54	40.57	37.13
	Phos.	42.51	44.27	37.45	43.49	45.10	38.94
	VAM	45.89	47.24	40.83	46.21	47.64	41.79
15.5	0.0	42.98	46.58	39.28	44.52	46.64	39.54
	Phos.	45.84	48.84	42.68	45.94	49.05	42.48
	VAM	48.35	50.48	44.72	49.13	51.49	45.89
23.25	0.0	46.25	49.58	44.28	46.48	49.94	43.56
	Phos.	50.52	52.64	46.25	51.03	53.23	46.58
	VAM	55.65	55.16	50.21	55.45	55.86	50.46
31	0.0	50.43	52.45	48.52	52.00	53.98	47.91
	Phos.	54.65	54.25	49.25	53.65	54.45	48.38
	VAM	56.12	55.53	50.54	55.85	56.12	50.86
LSD at 0.05		4.29	2.97	3.69	4.42	4.25	3.34

Bio: Bio-fertilizers VAM : Vesicular Arbuscular Mycorrhizae Phos.: Phosphorein

The combination between the highest level of mineral phosphate (31kg P_2O_5 / fed) and bio-fertilizers (phosphorein and VA-Mycorrhizae) fertilized plants resulted in the highest plant height and number of branches at all three cuts in the two seasons. Also, the combination between phosphate at 23.25 Kg P_2O_5 / fed and bio-fertilizers had a higher significant effect on the vegetative growth characters, whereas the untreated plants (control) gave the lowest values, in all cuts of both seasons. The data showed, in general, few differences among the means of the various fertilizer treatments. The increase in plant height and number of branches due to the VAM inoculation may be referred to enhancing some physiological processes in the plant roots, stimulated bud formation and consequently may increase lateral branching habit in treated plants (Gea *et al*., 1994). These results go well with those of

Abd El-Latif (2002) on caraway, EL-Ghadban *et al*. (2003) on marjoram and Mohamed & Saad (2004) on yarrow.

Table (2) : Effect of mineral and bio-phosphate fertilizers and their combination on number of branches / plant of marjoram plant during 2004 and 2005 seasons for three cuts.

Treatments		Number of branches / plant					
		First season			Second season		
P_2O_5 (kg / fed)		1st cut	2nd cut	3rd cut	1st cut	2nd cut	3rd cut
0.0		8.22	11.00	17.44	8.00	12.33	16.33
15.5		9.22	11.78	19.33	9.00	13.67	17.22
23.25		10.22	12.78	21.11	10.00	14.55	17.89
31		10.56	13.33	22.45	10.67	14.89	19.56
LSD at 0.05		1.15	0.90	1.23	0.94	2.12	1.54
Bio-phosphate							
0.0		8.59	11.25	18.67	8.75	13.17	17.09
Phosphorein		9.67	11.92	20.25	9.42	13.75	17.58
VAM		10.42	13.50	21.34	10.09	14.67	18.59
LSD at 0.05		1.02	0.60	1.12	0.57	1.24	1.32
Interaction							
0.0	0.0	6.67	10.33	16.33	7.33	11.67	15.67
	Phos.	8.33	11.00	17.67	8.00	12.33	16.33
	VAM	9.67	11.67	18.33	8.67	13.00	17.00
15.5	0.0	8.67	11.33	18.00	8.33	12.67	16.67
	Phos.	9.33	11.67	19.33	9.00	13.67	17.00
	VAM	9.67	12.33	20.67	9.67	14.67	18.00
23.25	0.0	9.33	11.33	19.33	9.33	14.00	17.33
	Phos.	10.33	12.33	21.33	10.00	14.33	17.67
	VAM	11.00	14.67	22.67	10.67	15.33	18.67
31	0.0	9.67	12.00	21.00	10.00	14.33	18.67
	Phos.	10.67	12.67	22.67	10.67	14.67	19.33
	VAM	11.33	15.33	23.67	11.33	15.67	20.67
LSD at 0.05		2.04	1.21	2.23	1.14	2.47	2.64

Bio: Bio-fertilizers VAM : Vesicular Arbuscular Mycorrhizae Phos.: Phosphorein

2- Plant weight :

Data presented in Tables (3 and 4) revealed that P fertilization at the highest rate (31Kg P_2O_5 / fed) significantly increased herb fresh weight 577.5, 569.0 g / plant and herb dry weight 136.0, 132.9 g / plant respectively, as total cuts in both seasons compared with control.

The combination between the highest rate of P fertilization and bio-phosphate produced higher herb fresh weight (575.7 and 564.9 g / plant) and herb dry weight (135.4, 132.1 g / plant) respectively, as total cuts in both seasons. The positive effect of phosphorus may be due to the role of phosphorus in photosynthes and respiration in addition to its role in cell division and development of merestematic tissues (Mengel and Kirkby, 1996). The increases in plant fresh and dry weight may be related to active bacteria in phosphorein, which is capable to convert tricalcium phosphate to monocalcium phosphate (Sherief *et al*., 1997). These results are ingreement with Shalan *et al*. (2001) on chamomile and Helmy (2003) on roselle.

Results presented in Tables (3 and 4) also showed that the combination between the highest rate of mineral phosphate and VA-Mycorrhizae had the superiority to increase marketable herb fresh weight (591.0, 590.3 g / plant) and dry weight (139.2, 137.5 g / plant) respectively, in the two seasons as total cuts. Such result may be attributed to the main role of VAM fungi to achive maximum growth by increasing uptake of soil phosphate more than other nutrients and stimulation of rapid growth may thus occur. The increase in plant fresh and dry weight may be attributed to phosphorus uptake and increased P percentage in plant tissues.

The considerable improvement by VAM fungi in the production of enzymes may enhance respiration of the host root (Mohamed and Saad, 2004). These results coincided with Kandeel and Sharaf (2003) who reported that, plants inoculation with VA-Mycorrhizae significantly increased fresh and dry weight of marjoram plant.

Table (3) : Effect of mineral and bio-phosphate fertilizers and their combination on herb fresh weight (g / plant) of marjoram plant during 2004 and 2005 seasons for three cuts.

Treatments		Herb fresh weight (g / plant)							
		First season				Second season			
P_2O_5 (kg / fed)		1st cut	2nd cut	3rd cut	Total cuts	1st cut	2nd cut	3rd cut	Total cuts
0.0		132.1	196.4	170.7	499.2	136.0	210.3	168.1	514.4
15.5		138.9	204.6	181.8	525.4	140.5	217.7	180.6	538.7
23.25		145.2	217.0	194.3	556.5	146.2	223.1	188.7	557.9
31		148.6	225.1	203.8	577.5	150.1	226.0	192.9	569.0
LSD at 0.05		8.4	3.7	4.8	9.6	4.7	6.5	5.9	10.9
Bio-phosphate									
0.0		135.9	205.0	181.0	521.9	139.4	212.8	174.3	526.5
Phosphorein		123.5	192.2	165.3	481.0	131.1	200.1	159.5	490.7
VAM		146.8	218.6	194.5	559.8	147.1	227.0	191.8	566.0
LSD at 0.05		4.9	2.8	4.2	8.1	3.4	5.2	6.1	10.3
Interaction									
0.0	0.0	123.5	192.2	165.3	481.0	131.1	200.1	159.5	490.7
	Phos.	132.2	196.1	170.5	498.8	136.2	211.5	168.4	516.1
	VAM	140.5	200.9	176.4	517.8	140.8	219.2	176.3	536.3
15.5	0.0	133.6	198.1	172.8	504.5	137.0	213.6	171.5	522.1
	Phos.	138.7	201.3	180.2	520.2	139.4	215.1	180.7	535.2
	VAM	144.4	214.5	192.5	551.4	145.0	224.3	189.6	558.9
23.25	0.0	140.5	209.7	186.2	536.4	141.5	217.5	182.1	541.1
	Phos.	145.2	212.8	196.3	554.3	146.9	220.7	186.7	554.3
	VAM	149.8	228.6	200.5	578.9	150.1	231.1	197.2	578.4
31	0.0	146.1	219.8	199.8	565.7	148.0	219.8	184.1	551.9
	Phos.	147.2	225.3	203.2	575.7	149.7	224.7	190.5	564.9
	VAM	152.4	230.2	208.4	591.0	152.6	233.5	204.2	590.3
LSD at 0.05		9.7	5.6	8.4	16.2	6.8	10.4	12.2	20.6

Bio: Bio-fertilizers VAM : Vesicular Arbuscular Mycorrhizae Phos.: Phosphorein

Table (4) : Effect of mineral and bio-phosphate fertilizers and their combin ation on herb dry weight (g / plant) of marjoram plant during 2004 and 2005 seasons for three cuts.

Treatments		Herb dry weight (g / plant)							
		First season				Second season			
P_2O_5 (kg / fed)		1st cut	2nd cut	3rd cut	Total cuts	1st cut	2nd cut	3rd cut	Total cuts
0.0		29.6	43.6	37.9	111.2	29.3	45.1	34.0	106.5
15.5		31.8	47.6	42.5	121.8	31.5	48.3	39.6	117.4
23.25		33.8	50.9	45.7	130.4	35.1	50.6	43.6	127.3
31		34.5	53.0	48.5	136.0	36.4	52.7	45.8	132.9
LSD at 0.05		2.4	1.1	1.1	3.3	1.1	1.5	1.9	4.5
Bio-phosphate									
0.0		31.2	47.3	42.1	120.6	32.1	47.6	38.6	116.3
Phosphorein		32.2	48.4	43.7	124.3	33.1	48.8	40.8	120.7
VAM		34.0	50.6	45.2	129.8	34.1	51.2	42.8	126.1
LSD at 0.05		1.8	0.9	1.0	2.1	0.8	1.2	1.8	2.1
Interaction									
0.0	0.0	27.5	42.7	36.7	106.9	28.3	42.7	31.8	100.8
	Phos.	29.5	43.6	37.9	111.0	29.4	45.1	34.3	106.8
	VAM	31.9	44.6	39.2	115.7	30.3	47.6	36.0	111.9
15.5	0.0	30.4	46.1	40.2	116.7	30.7	46.4	36.5	111.6
	Phos.	31.4	46.8	42.4	120.6	31.3	47.7	40.1	117.1
	VAM	33.6	49.8	44.8	128.2	32.5	50.8	42.2	123.5
23.25	0.0	32.7	48.8	43.8	125.3	33.9	49.3	42.4	123.6
	Phos.	33.8	50.1	46.2	130.1	35.2	50.1	43.4	126.7
	VAM	34.9	53.8	47.2	135.9	36.1	52.4	45.0	131.5
31	0.0	34.0	51.7	47.6	133.3	35.4	52.0	43.8	129.2
	Phos.	34.0	53.0	48.4	135.4	36.6	52.1	45.4	132.1
	VAM	35.4	54.2	49.6	139.2	37.3	54.1	48.1	137.5
LSD at 0.05		3.6	1.8	2.0	4.2	1.6	2.4	3.6	4.2

Bio: Bio-fertilizers VAM : Vesicular Arbuscular Mycorrhizae Phos.: Phosphorein

3- Essential oil productivity :

The essential oil percentage and content in the dried leaves of marjoram varied due to phosphate fertilizer treatments (Table, 5). Highest oil percentages were (1.71, 1.63 and 1.51 %) and (1.69, 1.63 and 1.43 %) respectively, in the three cuts in the first and the second seasons. These values were obtained from plants fertilized with the highest phosphate level (31 kg P_2O_5 / fed).

Concerning the effect of inoculated plants with phosphorein and VA-Mycorrhizae, there were significant increases on the plant essential oil percentage and content. Similar results of positive effect of phosphorein and VA-Mycorrhizae on the essential oil productivity were obtained by Shalan *et al.* (2001) on chamomile, Abdel-Kader & Ghaly (2003) on coriander, Kandeel & Sharaf (2003) on marjoram and Mohamed & Saad (2004) on yarrow.

The combination between the high level of mineral phosphate fertilization and bio-phosphate caused an increase in the essential oil percentage (1.70, 1.63 and 1.50 % respectively) and (1.69, 1.64 and 1.44 % respectively) of the three cuts at both seasons. In the same Table P fertilization at the highest rate (31 kg P_2O_5 / fed) combination with Mycorrhizae had increased marketable oil percentage (1.75, 1.65 and 1.53 %, respectively) and (1.71, 1.65 and 1.45 %, respectively) in the three cuts at both seasons. These increases might be attributed to the enhancing effect on vegetative growth, in terms of fresh yield and increased uptake of nutrients by root of plant especially phosphorus element. However, the most important compound in phosphate group which linked by pyrophosphate bonds is adenosine triphosphate (ATP). In this form, the energy can be conveyed to various undergoing processes such as activation uptake and the synthesis of various organic compounds such as essential oil (El-Ghadban *et al*., 2003).

Table (5): Effect of mineral and bio-phosphate fertilizers and their combination on essential oil percentage / plant of marjoram plant during 2004 and 2005 seasons for three cuts.

Treatments		Essential oil percentage / plant (%)					
		First season			Second season		
P_2O_5 (kg / fed)		1st cut	2nd cut	3rd cut	1st cut	2nd cut	3rd cut
0.0		1.64	1.55	1.46	1.63	1.55	1.41
15.5		1.68	1.59	1.48	1.65	1.60	1.42
23.25		1.69	1.62	1.50	1.67	1.62	1.43
31		1.71	1.63	1.51	1.69	1.63	1.43
LSD at 0.05		0.01	0.02	0.01	0.02	0.01	0.02
Bio-phosphate							
0.0		1.65	1.57	1.47	1.63	1.58	1.41
Phosphorein		1.68	1.60	1.49	1.66	1.61	1.43
VAM		1.72	1.62	1.51	1.69	1.62	1.44
LSD at 0.05		0.01	0.01	0.01	0.02	0.01	0.01
Interaction							
0.0	0.0	1.61	1.52	1.44	1.59	1.54	1.39
	Phos.	1.64	1.54	1.46	1.63	1.55	1.41
	VAM	1.68	1.58	1.48	1.66	1.57	1.42
15.5	0.0	1.65	1.56	1.46	1.63	1.57	1.40
	Phos.	1.68	1.59	1.48	1.65	1.60	1.42
	VAM	1.71	1.61	1.51	1.68	1.62	1.44
23.25	0.0	1.66	1.59	1.47	1.65	1.60	1.41
	Phos.	1.68	1.62	1.50	1.66	1.63	1.43
	VAM	1.74	1.65	1.52	1.71	1.64	1.46
31	0.0	1.67	1.62	1.49	1.66	1.61	1.43
	Phos.	1.70	1.63	1.50	1.69	1.64	1.44
	VAM	1.75	1.65	1.53	1.71	1.65	1.45
LSD at 0.05		0.02	0.02	0.02	0.04	0.02	0.02

Bio: Bio-fertilizers VAM : Vesicular Arbuscular Mycorrhizae Phos.: Phosphorein

In addition data presented in Table (6) revealed that the essential oil content markedly increased as P rate were increased 2.19 and 2.14 ml / plant as a total cuts in both seasons compared with the control plants.These values were obtained from plants fertilized with the highest phosphate level (31 kg P_2O_5 / fed).

Moreover results presented in Table (6) showed the combination between mineral phosphate, phosphorein and VA-Mycorrhiza on oil content. It was observed that the highest values were recorded due to the treatment of high level of phosphorus (31 Kg P2O5 / fed) combined with VA-Mycorrhizae (2.27 and 2.23 ml / plant) as a total cuts in both seasons. Also, the combination between the treatment at 23.25 Kg P2O5 / fed plus VA-Mycorrhizae had a higher oil content (2.22 and 2.14 ml / plant). The same trend was observed from the combination between mineral and bio phosphate at the same treatments. These results may be a consequent of the increase in plant fresh and dry weight as a result of bio-fertilization, as well as, the increase in the essential oil percentage.

Table (6): Effect of mineral and bio-phosphate fertilizers and their combination on essential oil content (ml / plant) of marjoram plant during 2004 and 2005 seasons for three cuts.

Treatments		Essential oil content (ml / plant)							
		First season				Second season			
P_2O_5 (kg / fed)		1st cut	2nd cut	3rd cut	Total cuts	1st cut	2nd cut	3rd cut	Total cuts
0.0		0.49	0.67	0.55	1.71	0.48	0.70	0.48	1.65
15.5		0.53	0.75	0.63	1.92	0.52	0.77	0.57	1.87
23.25		0.57	0.83	0.68	2.08	0.59	0.83	0.63	2.04
31		0.59	0.86	0.73	2.19	0.62	0.86	0.66	2.14
LSD at 0.05		0.03	0.02	0.01	0.10	0.03	0.03	0.02	0.09
Bio-phosphate									
0.0		0.51	0.75	0.62	1.88	0.53	0.76	0.55	1.83
Phosphorein		0.54	0.77	0.65	1.96	0.55	0.79	0.59	1.92
VAM		0.59	0.82	0.69	2.09	0.58	0.83	0.62	2.03
LSD at 0.05		0.02	0.01	0.01	0.09	0.02	0.02	0.01	0.07
Interaction									
0.0	0.0	0.44	0.65	0.53	1.62	0.45	0.66	0.44	1.55
	Phos.	0.48	0.67	0.55	1.70	0.48	0.70	0.48	1.66
	VAM	0.54	0.70	0.58	1.82	0.50	0.74	0.51	1.75
15.5	0.0	0.50	0.72	0.59	1.81	0.50	0.73	0.51	1.74
	Phos.	0.53	0.74	0.63	1.90	0.52	0.76	0.60	1.88
	VAM	0.57	0.80	0.68	2.05	0.55	0.82	0.61	1.98
23.25	0.0	0.54	0.78	0.63	1.95	0.56	0.79	0.60	1.95
	Phos.	0.57	0.81	0.69	2.07	0.58	0.83	0.62	2.03
	VAM	0.61	0.89	0.72	2.22	0.62	0.86	0.66	2.14
31	0.0	0.57	0.84	0.71	2.12	0.59	0.84	0.63	2.06
	Phos.	0.59	0.86	0.73	2.18	0.62	0.85	0.65	2.12
	VAM	0.62	0.89	0.76	2.27	0.64	0.89	0.70	2.23
LSD at 0.05		0.04	0.02	0.02	0.18	0.04	0.04	0.02	0.14

Bio: Bio-fertilizers AM : Vesicular Arbuscular Mycorrhizae Phos.: Phosphorein

4- Essential oil components :

Data presented in Table (7) and illustrated in Figure (1) identified 9 compounds formed from 96.2 to 99.3 % of the oil, indicating the effect of mineral and bio-phosphate fertilization and their combination.

Cineole was the major constituent forming from 12.1 to 42.9 %, followed by Linalyl acetate (2.8 - 33.8 %), Carvacrol (9.4 - 32.1 %), Limonene (6.9 - 11.8 %), α-Terpinene (3.5 - 8.4 %), Geranyl-acetate (2.6 - 7.4 %), β-pinene (1.0 - 5.9 %), methyl chavicol (1.7 - 5.8 %) and α-pinene (0.8 - 2.5 %).

It is evident from the results that phosphorus fertilization increased the percentages of carvacrol, α-terpinene, α-pinene components in marjoram oil. The highest percentage of β-pinene 5.9% was obtained from the highest levels of phosphorus 31 Kg P2O5 / fed.

The inculation with VAM-Mycorrhizae increased the percentage of carvacrol and methyl chavicol.

Concerning the effect of the combination between the P fertilizer at the rate of 15.5 Kg P2O5 / fed had the highest percentage of Linalyl acetate 33.8 % of marjoram oil. The highest value of cineole (42.9 %) and limonene (11.8 %) were produced from the highest level of phosphorus fertilizer and VAM fungi combination. The constructive effect may be due to VAM fungi which could encourage plant growth, absorption of nutrients (N, P, K, Mn, Zn and Fe), efficiency of nutrient and the metabolism of photosynthesis (Reynders & Vlassak, 1982).

Table (7) : Effect of mineral and bio-phosphate fertilizers and their combination on the essential oil components (%) of marjoram plant from the second cut at 2005 season.

Treatments		Essential oil components (%)								
A	B									
P_2O_5 (Kg/fed)	Bio	α-Pinene	β-Pinene	Lemonene	Cineole	Linalyl acetate	Methyl chavicol	Carvacrol	Garanyl acetate	α-Terpinen
0	0	2.5	1.0	6.9	23.5	12.8	4.8	29.8	6.5	8.4
	Phos.	2.0	1.5	7.5	23.9	13.7	4.9	31.4	5.4	7.8
	VAM	1.9	1.9	8.5	23.8	15.2	5.7	32.1	5.6	3.6
15.5	0	1.6	1.4	8.8	18.9	25.4	4.1	29.2	5.1	3.5
	Phos.	1.4	2.8	9.8	13.6	33.3	1.7	25.3	7.4	3.5
	VAM	0.8	2.9	10.8	12.7	33.8	1.8	25.7	6.5	3.7
23.25	0	1.0	3.1	11.2	12.1	30.2	2.5	27.8	7.1	4.0
	Phos.	1.0	3.2	11.6	30.6	20.4	5.4	20.0	2.6	4.3
	VAM	1.6	4.2	11.5	35.4	20.7	5.8	12.9	3.4	3.7
31	0	1.5	5.9	10.5	41.9	16.8	4.8	9.4	3.2	5.2
	Phos.	1.8	5.1	10.8	42.0	16.2	4.7	10.3	3.8	4.6
	VAM	1.1	4.9	11.8	42.9	16.5	4.8	9.7	3.2	4.4

Bio: Bio-fertilizers   VAM : Vesicular arbuscular mycorrhizae   Phos.: Phosphorein

These results agreed with Clark and Mematy (1981) on peppermint indicated that, photosynthesis is an important determinate of essential oil composition. Also, they came to the conclusion that, physiological factors may significantly affect the composition of terpenes produced in any given environment.

5- Herb and oil yield

Data in Table (8) revealed significant differences in dried herb yield due to different levels of phosphorus fertilizer (0, 15.5, 23.25 and 31 kg P_2O_5 / fed).

Figure (1): Effect of mineral and bio-phosphate fertilizers and their combination on the essential oil components (%) of marjoram plant during 2005 season.

Fertilization with the highest level of mineral P produced the heaviest herb yield 3.02 and 2.95 ton / fed respectively, in the both seasons compared with the control plants. Concerning the effect of bio-fertilizer (phosphorein and Mycorrhizae) increased yearly herb yield.

In regard to the combination effect, the obtained data indicated that heaviest yearly herb yield was obtained from plants fertilized with P at the rate of 31 kg P_2O_5 / fed plus Mycorrhizae 3.09 and 3.05 ton / fed in both seasons. These results agreed with El-Ghadban *et al*. (2003), Kandeel and Sharaf (2003) on marjoram.

Data presented in Table (8) showed that different levels of P fertilizer had significant differences in essential oil yield. Fertilization with the highest level of mineral phosphate produced the highest yearly essential oil yield 48.66 and 47.48 liter / fed respectively, in the both seasons. Meanwhile, the control plants was significantly the lowest.

Table (8): Effect of mineral and bio-phosphate fertilizers and their combination on yearly dried herb and essential oil yield / fed of marjoram plant during 2004 and 2005 seasons.

Treatments		Yearly dried herb yield (ton / fed)		Yearly essential oil yield (liter / fed)	
P_2O_5 (kg / fed)		1st season	2nd season	1st season	2nd season
0.0		2.47	2.37	38.07	36.73
15.5		2.71	2.61	42.74	41.48
23.25		2.89	2.83	46.22	45.33
31		3.02	2.95	48.66	47.48
LSD at 0.05		0.12	0.11	2.02	1.98
Bio-phosphate					
0.0		2.68	2.59	41.72	40.55
Phosphorein		2.76	2.68	43.61	42.72
VAM		2.88	2.80	46.44	45.00
LSD at 0.05		0.11	0.09	1.58	1.39
Interaction					
0.0	0.0	2.38	2.24	35.99	34.44
	Phos.	2.46	2.37	37.77	36.88
	VAM	2.57	2.49	40.44	38.88
15.5	0.0	2.59	2.48	40.44	38.66
	Phos.	2.68	2.60	42.22	41.77
	VAM	2.85	2.74	45.55	44.00
23.25	0.0	2.78	2.75	43.33	43.33
	Phos.	2.89	2.82	46.00	45.11
	VAM	3.01	2.92	49.33	47.55
31	0.0	2.96	2.87	47.11	45.77
	Phos.	3.01	2.93	48.44	47.11
	VAM	3.09	3.05	50.44	49.55
LSD at 0.05		0.22	0.18	3.15	2.77

Bio: Bio-fertilizers VAM : Vesicular Arbuscular Mycorrhizae Phos.: Phosphorein

Concerning the effect of bio-fertilizer (phosphorein and Mycorrhizae) increased yearly oil yield. In regard to the combination effect, the obtained data indicated that highest oil yield was obtained from plants fertilized with P rate of 31 kg P_2O_5 / fed plus Mycorrhizae (50.44 and 49.55 liter / fed) in both seasons. The increase in essential oil yield per feddan may be due to the increase in yearly herb yield, as well as the increament in the oil percentage as a result of inoculating plants by phosphorein and VAM fungi with the different levels of mineral phosphorus. These results are in harmony with those obtained by El-Ghadban *et al*.(2003)on marjoram

6- Mineral elements :

Data presented in Table (9) indicated that mean values of N, P and K contents of marjoram were significant increased with increasing rates of mineral P fertilizer from 15.5 up to 31Kg P2O5 / fed, compared with the untreated ones during the two experimental seasons. The highest values of N, P and K percentages were produced from treated plants with the highest level of phosphorus fertilization.

Table (9): Effect of mineral and bio-phosphate fertilizers and their combination on the percentages of mineral elements N, P and K (%) of the marjoram plant during 2004 and 2005 seasons for three cuts.

Treatments		Mineral elements percentages (%)								
		N %			P %			K %		
P_2O_5 (Kg/ fed)	Bio	First season								
		1st cut	2nd cut	3rd cut	1st cut	2nd cut	3rd cut	1st cut	2nd cut	3rd cut
0.0	0	2.54	2.61	2.74	0.48	0.52	0.57	2.78	2.84	2.81
	Phos.	2.56	2.64	2.76	0.49	0.54	0.59	2.81	8.86	2.84
	VAM	2.58	2.66	2.78	0.51	0.57	0.62	2.86	2.87	2.86
15.5	0	2.56	2.65	2.77	0.51	0.57	0.60	2.81	2.87	2.85
	Phos.	2.58	2.67	2.80	0.54	0.61	0.64	2.94	2.89	2.87
	VAM	2.63	2.69	2.85	0.58	0.65	0.66	2.95	2.91	2.89
23.25	0	2.59	2.69	2.83	0.55	0.62	0.66	2.97	2.91	2.88
	Phos.	2.62	2.72	2.86	0.58	0.64	0.68	2.95	2.95	2.92
	VAM	2.64	2.74	2.88	0.63	0.68	0.71	2.98	2.98	2.94
31	0	2.60	2.73	2.86	0.57	0.66	0.69	0.96	2.94	2.96
	Phos.	2.63	2.75	2.88	0.59	0.67	0.71	2.98	2.96	2.97
	VAM	2.67	2.75	2.93	0.64	0.69	0.72	2.99	2.98	2.98
Second season										
0.0	0	2.59	2.65	2.79	0.53	0.59	0.58	2.74	2.82	2.85
	Phos.	2.62	2.68	2.84	0.54	0.62	0.59	2.79	8.84	2.88
	VAM	2.64	2.69	2.86	0.57	0.64	0.64	2.83	2.89	2.89
15.5	0	2.61	2.69	2.86	0.55	0.62	0.61	2.79	2.88	2.87
	Phos.	2.64	2.73	2.89	0.59	0.65	0.65	2.85	2.89	2.89
	VAM	2.66	2.78	2.92	0.61	0.68	0.68	2.89	2.93	2.92
23.25	0	2.65	2.77	2.90	0.59	0.66	0.66	2.89	2.92	2.98
	Phos.	2.68	2.84	2.92	0.62	0.67	0.68	2.93	2.95	2.93
	VAM	2.72	2.86	2.94	0.65	0.69	0.69	2.96	2.98	2.96
31	0	2.72	2.87	2.91	0.63	0.69	0.68	2.95	2.95	2.95
	Phos.	2.74	2.86	2.93	0.64	0.70	0.69	2.97	2.97	2.97
	VAM	2.76	2.87	2.95	0.64	0.71	0.70	2.98	2.99	2.98

Bio: Bio-fertilizers VAM : Vesicular Arbuscular Mycorrhizae Phos.: Phosphorein

Also, data in the same Table indicated that plants fertilized by 31 kg P_2O_5 / fed combined with VA-Mycrohizea caused an increase in N, P and K percentages in the three cuts during both seasons when compared with the untreated ones. The combination between phosphorus levels at the rate of 15.5 to 31 Kg P2O5 / fed with phosphorein and Mycorrhizae had no significant effect on N, P and K contents of marjoram leaves. Similar results were obtained by Helmy (2003) on roselle and Kandeel & Sharaf (2003) on marjoram.

The effect of mineral and bio-phosphorus on total bacteria (P-solubilizing) and spores of Vesicular Arbuscular Mycorrhizae (VAM) fungi counts were investigated. Data presented in Table (10) showed that the introduced phosphate dissolving bacteria and spore of VAM fungi were able to actively colonize the rhizosphere of marjoram plants when compared with uninoculated ones. The density phosphate dissolving bacteria an spore of VAM fungi were increased in the rhizosphere of marjoram plants with increase phosphorus fertilizer level. The population size of tested P-dissolving bacteria and spore of VAM fungi indicated an active rhizosphere of VAM fungi and were found in most phosphorus and inoculation combinations.

The treatments fertilized with 31 Kg P2O5 / fed was most active followed by the treatments with 23.25 Kg P2O5 / fed, then 15.5 Kg P2O5 / fed. These findings are in harmony with Abdel-Fattah and Shabana (2000) on cowpen plants.

Table (10) : Effect of mineral and bio-phosphate fertilizers and their combination on number of phosphate solubilizing bacteria in soil of marjoram plant at 2005 season.

Treatments		Counts spore of VAM fungi (x 10^5 cell / g soil)			Counts of P-solubizing bacteria (x 10^5 cell / g soil)		
A	B	Second season					
P_2O_5 (Kg/fed)	Bio	1st cut	2nd cut	3rd cut	1st cut	2nd cut	3rd cut
0	0	0.02	0.06	0.07	1.02	1.24	1.68
	Phos.	o.05	0.07	0.07	3.24	2.94	3.25
	VAM	0.75	1.04	1.05	2.51	1.97	2.01
15.5	0	0.03	0.07	0.09	1.45	1.78	1.81
	Phos.	0.06	0.07	0.12	6.58	5.83	6.73
	VAM	1.21	2.04	2.04	3.85	2.45	3.42
23.25	0	0.06	0.09	0.09	2.58	2.01	2.09
	Phos.	0.06	0.11	0.21	7.81	6.78	7.82
	VAM	2.09	2.91	3.01	3.98	2.31	3.15
31	0	0.08	1.02	1.02	2.86	2.54	3.52
	Phos.	0.07	1.01	1.01	8.12	7.86	8.34
	VAM	2.52	3.04	3.04	6.21	3.12	3.72

Bio: Bio-fertilizers VAM : Vesicular Arbuscular Mycorrhizae Phos.: Phosphorein

The aforementioned results of the present study, recommended to fertilize marjoram plants with the combination of 31 Kg P2O5 / fed with VA-Mycorrhizae to produce high yield of herb with good essential oil quality.

Also, using bio-fertilizers may be considered as a substitute and/or to decrease the mineral fertilizers application and consequently reduce the environment pollution.

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تـأثير التسـميد الفوسـفاتى المعـدنى والحيـوى علـى النمـو وإنتـاج الزيـت الطيـار والمكونات الكيماوية لنبات البردقوش

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أجريت هذه التجربة خلال موسمى الزراعة ٢٠٠٤ و٢٠٠٥ فى مزرعة النباتات الطبية و العطرية بكلية الزراعة- جامعة المنصورة بهدف دراسة تأثير مستويات مختلفة من سماد السوبرفوسفات (صفر- ١٥,٥ - ٢٣,٢٥ -٣١ كجم فو٢ أ٥ / فدان) مع أو بدون السماد الحيوى (فوسفورين – ميكروهيزا) على النمو الخضرى ومحصول العشب و محتوى الزيت الطيار وأيضا المكونات الكيماوية لنبات البردقوش.

أوضحت النتائج أن النباتات المعاملة بالسوبر فوسفات أعطت زيادة معنوية واضحة فى صفات النمو ومحصول العشب ومحتوى الزيت الطيار والعناصر الكيماوية حيث كانت أفضل النتائج عند إستخدام المستوى الأعلى بتركيز (٣١ كجم فو٢ أ٥ / فدان).

أدت المعاملة بالسماد الحيوى (ميكروهيزا أو الفوسفورين) الى تحسين صفات النمو وكذا محتوى الزيت الطيار وأيضا العناصر الكيماوية بالمقارنه بمعاملة الكونترول.

أدى التفاعل بين الفوسفات المعدنى والحيوى الى حدوث زيادة معنوية فى صفات النمو ومحصول العشب ومحتوى الزيت الطيار و أيضا العناصر الكيماوية للنباتات المعاملة مقارنة بالمستويات المستخدمة من السماد المعدنى الفسفورى (٢٣,٢٥ ، ٣١ كجم فو٢ أ٥ / فدان) بينما كانت أفضل النتائج من النباتات المعاملة بالميكروهيزا عند مستوى ٣١ كجم / فدان من سماد السوبر فوسفات.

توصى النتائج سالفة الذكر آنه للحصول على أكبر محصول من العشب به نوعية جيدة من الزيت الطيار يفضل تسميد نبات البردقوش بمخلوط من السماد المعدنى والحيوى. حيث يؤدى ذلك لتقليل إستخدام الأسمدة المعدنية كما يقلل من تلوث البيئة.