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Effect of Biofertilization and Organic Manuring on Soil Dehydrogenase Activity, Macronutrients and Essential Oil Content of Marjoram

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TWO FIELD experiments were carried out at an organic farm (Minoufia Governorate) during 2005- 2006 and 2006-2007 seasons to study the interaction effect of biofertilization and organic manuring on dehydrogenase, polyphenol oxidase and peroxidase activity, macronutrients uptake, oil yield and oil composition of marjoram. Greater activities of dehydrogenase, peroxidase and polyphenol oxidase were obtained in soil and marjoram treated with combination of biofertilizers and organic manure than soil treated with biofertilizers or organic manure. The greatest activities of peroxidase and polyphenol oxidase were obtained with the treatment of biofertilization combined with full dose of organic manure. Similar results trend was observed with N, P and K uptake because N, P and K uptake by marjoram were greater with dual application of biofertilization and organic manure than other treatments. There is no significant difference between oil yield obtained with chemical fertilization treatment and biofertilizers combined with full dose of organic manure treatment. Fourteen compounds, accounting for more than 97 % of the total volatiles components in most marjoram samples were detected and identified. Dual application of organic manure and biofertilization to soil and marjoram produced maximum percent of terpinen-4-ol (the major compound in marjoram oil) compound in marjoram.

Keywords: Marjoram, Biofertilization, Organic manuring, *T. Harzianum*, Dehydrogenase, Macronutrients uptake, Marjoram essential oil, Yield and oil composition.

Marjoram (*Majorana hortensis*) is known to the ancient Egyptians and has been used as a food flavour and medicinal herb. Essential oil has been known since antiquity to possess biological activity, notably antibacterial, antifungal as well as antioxidant properties (Tiziana & Dorman, 1998). El-Ghadban *et al*. (2002) reported that marjoram treatment with compost and biofertilizer led to an increase in macro-nutrients uptake. These increases might be related to the synergistic effects of compost and microorganisms on micro-nutrients production and availability to crops. Application of biofertilizers (*Azotobacter chroococcum*, *Azospirillum lipoferum*, *Paenibacillus polymyxa* and *Bacillus megaterium*) increased nitrogen, phosphorus and potassium content in marjoram herb (Mahfouz, 2003).

Biofertilizers consist of beneficial microorganisms which accelerate and improve plant growth and protect plants from pests and diseases. (Abou-Aly *et al*. (2006). To overcome the ecological problems resulting from the loss of plant nutrients and to increase crop yield, microorganisms may enhance availability of nutrients to plants and provide sustainable solutions for present and future agricultural practices. Gailite *et al*. (2005) reported that the content of both peroxidase and polyphenol oxidase activities in bean leaves increased following cultivation in the presence of plant growth promoting rhizobacteria. In addition, the essential oil components of rosemary were increased with the application of biofertilizers (Leithy *et al*., 2006). Combined treatment of soil with biofertilizers and compost gave better oil constituents of marjoram than those obtained from either N_2 fixers (*Azospirillum brasiliense, Azotobacter chroococcum* and *P. polymyxa*), *B. circulans* or compost (Gharib *et al*. (2008). Azizi *et al*. (2008) reported that essential oil yield of chamomile was increased with the increase in compost dose.

Therefore, this research was carried out to study the effects of organic manure and biofertilizers on marjoram oil yield, oil composition and enzyme activities.

Materials and Methods

Two field experiments were carried out at organic farm in Minoufia Governorate, Egypt, during 2005/ 2006 and 2006/2007 seasons. This experiment was designed to study the interaction effect of organic farming agents on some enzymes activity and active substances of marjoram. Seeds of marjoram were obtained from Medicinal and Aromatic Research Dept., A.R.C., Ministry of Agriculture.

Experimental soil

Soil samples obtained from different field places were mixed. Soil samples were subjected to physical and chemical analyses as follows. Physical and chemical analyses are presented in Table 1.

The main characteristics of used compost which obtained from organic farm in Minoufia Governorate, Ghareeb Sons Farms, are given in Table 2.

TABLE 1. Mechanical and chemical analysis of the experimental soil.

Particle size distribution		Coarse sand %	6.59
		Fine sand %	27.64
		Silt %	12.60
		Clay %	53.17
Textural class			Clay
pH			8.03
Electrical conductivity (dS/m)			2.02
Organic matter %			2.12
Soluble cations meq / L		Ca^{++}	12.1
		Mg^{++}	5.8
		Na^{+}	0.44
		K^{+}	1.86
Soluble anions meq / L		HCO_3^{-}	4.40
		CO_3^{2-}	0
		Cl^{-}	11.2
		SO_4^{2-}	4.6
Total and available macronutrients (ppm)	N	total	1730
		available	53.64
	P	total	561.2
		available	130.94
	K	total	3500
		available	1612.8

TABLE 2. Chemical analysis of the compost used in this experiment. Compost was obtained from Ghareeb Sons Farms located in Minoufia Governorate .

Parameters	**Unit**	**Value**
pH	-	8.11
Electrical conductivity (1:5 extract)	dS/m	8.21
Organic matter	%	21.57
Organic-C	%	12.54
Total-N	%	1.21
C/N ratio	-	10.36
Total-P	%	0.91
NH_4- N	ppm	274.7
NO_3-N	ppm	50.1

Physical and chemical analyses of soil and compost were carried out according to the method described by Page *et al.* (1982).

Experimental design

Treatments were distributed in a randomized complete block design with three replicates. The experimental plot area was 10.5 m^2 (3 x 3.5 m). This experiment included the following treatments:

T1: Control (non-fertilized and non-inoculated).

T2: Chemical fertilization (recommended doses of chemical fertilization N, P and K).

T3: Biofertilization (*P. polymyxa* H1 + *B. megaterium var phosphaticum*+ *B. circulans*).
T4: Biofertilization+ half dose of compost.
T5: Biofertilization + 3/4 dose of compost.
T6: Biofertilization+ recommended dose of compost.
T7: Recommended dose of compost (8 ton/ fed).

The biocontrol agent of *Trichoderma harzianum* was added to all investigated treatments.

Inocula preparation

Inocula of *Paenibacillus polymyxa*, *Bacillus megaterium* var. *phosphaticum, Bacillus circulans* and *Trichoderma harzianum* were prepared in nutrient broth (Cross *et al*., 1968), Modified Bunt and Rovira broth (Abdel-Hafez,1966), Alexandrov (Zahra, 1969) and gliotoxin fermentation media (Brain & Hemming, 1945), respectively.

Cultivation process

Prior to cultivation, plant bed was prepared. Seeds of marjoram were sown in prepared seed beds on 15/10/2005 and 15/10/2006 of both study seasons. After 50-60 days, seedlings 10-15 cm tall were individually transplanted in experimental field.

Chemical fertilizers were supplemented with a full dose of inorganic nitrogen (50 kg N/fed) as ammonium sulphate, inorganic phosphorus (25 kg P_2O_5/fed) as super phosphate and potassium (40 kg K_2O/fed) as potassium sulphate .The chemical fertilizers were applied in two equal doses, *i.e.* at vegetative and flowering stages.

Compost was added as organic manure to the soil at a rate of recommended dose (8 ton/fed), ¾ dose and ½dose before planting.

Biofertilizers and biocontrol agent application

Except for control treatment, transplants of marjoram were inoculated by dipping the root system in mixture of cell suspension of *P. polymyxa H1* ($7x10^{11}$c.f.u./ml), *B. megatherium* var *phosphaticum* ($8.3x10^{11}$c.f.u./ml), *B. circulans* ($4.1x10^{11}$c.f.u./ml) and *T. harzianum* ($5x10^7$ spores/ml) for 60 min before transplanting. Sucrose solution (30%) was added as an adhesive agent prior to inoculation. The same prepared inocula were added to the soil with irrigated water (100/ml for plot) three times throughout the growing seasons.

Determinations

- Dehydrogenase activity (DHA) was assayed in soil according to Thalmann (1967).
- Nitrogen, phosphorus and potassium percentage were determined in shoots according to the method described by A.O.A.C. (1980), A.PH.A. (1992) and

Dewis & Freitas (1970), respectively, after that the micro-nutrients uptake per plant was calculated.

- Peroxidase and polyphenol oxidase in plant were determined according to the method described by Allam & Hollis (1972) and Matta & Dimond (1963), respectively.
- Essential oil of air dried herb was extracted by water distillation for 3 hr and then dried over anhydrous sodium sulphate and determined according to Guenther (1961). The oil yield per plant and feddan were calculated.

Analysis of essential oil composition of marjoram

Essential oil samples were analyzed by GC–MS in Institute of Ecological Chemistry and Waste Analysis Technical University, Braunschweig, Germany according to Vera & Chane-Ming (1999).

Statistical analysis.

Obtained data were statistically analyzed according to Gomez & Gomez (1984). For comparison between means, Duncan's multiple range test was used (Duncan, 1955).

Results and Discussions

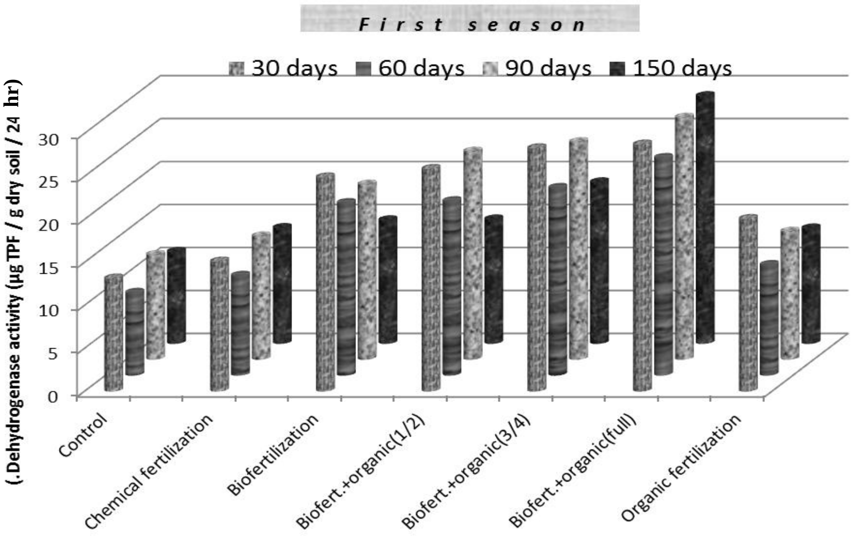
Effect of biofertilization and organic manuring on dehydrogenase activity of the soil

Data presented in Fig. 1 showed that DHA is widely varied among the studied treatments. Lower DHA was measured in soil treated with chemical fertilizers than soil treated with biofertilizers and/or compost. This result was in accordance with Krishnakumar *et al.* (2007) who reported that application of NPK fertilizers on soil reduces soil DHA significantly than soils treated with organic manure.

Higher records of DHA were observed in inoculated treatments inoculated with mixture of biofertilizer strains than soil treaded with organic manure only.

The higher records of DHA with biofertilization are likely be due to the effective role of inoculation for enhancing colonization of introduced biofertilizers for plant roots. Moreover, the inoculation might lead to the accumulation of available nutrients and stimulate the microorganisms in rhizosphere.

Moreover, data showed that soil treated with combination of biofertilizer strains and compost resulted in higher DHA than soil treated with either biofertilizers or compost each one individually. Similar trend of results was observed by Garciá-Gil *et al*. (2000) who reported that DHA was higher in organic manure treatments than soil treated with chemical fertilizer, indicating that increase in microbial activities may have occurred due to increase in available carbons from biodegradable organic manure.



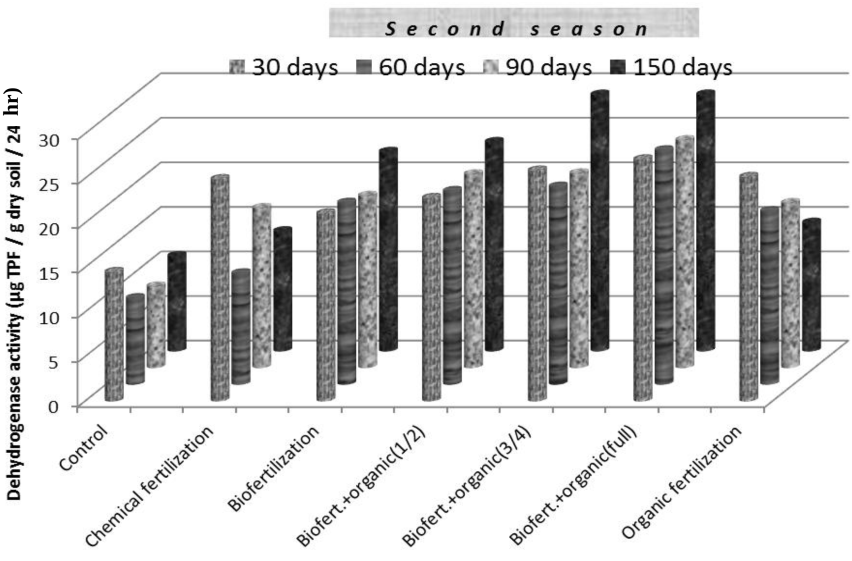


Fig. 1. Effect of biofertilization and organic manuring on dehydrogenase activity in soil cultivated with marjoram.

Data revealed that the soil treated with biofertilizer strains and *T. harzianum* combined with full dose of compost gave the highest values of DHA. The elevated DHA may be due to the effect of organic manure on growth of indigenous microorganisms and introduced biofertilizer strains and activities.

This result is in accordance with Balakrishnan *et al*. (2007) who found that the application of compost in combination with phosphate solubilizing bacteria significantly increased soil microflora such as bacteria, fungi and actinomycetes and soil enzyme activities such as dehydrogenase and phosphatase.

Effect of biofertilization and organic manuring on N, P and K uptake

Obtained data (Fig. 2) showed that N,P and K uptake significantly increased in plants grown in soil amended with compost than soil treated with biofertilizer strains.

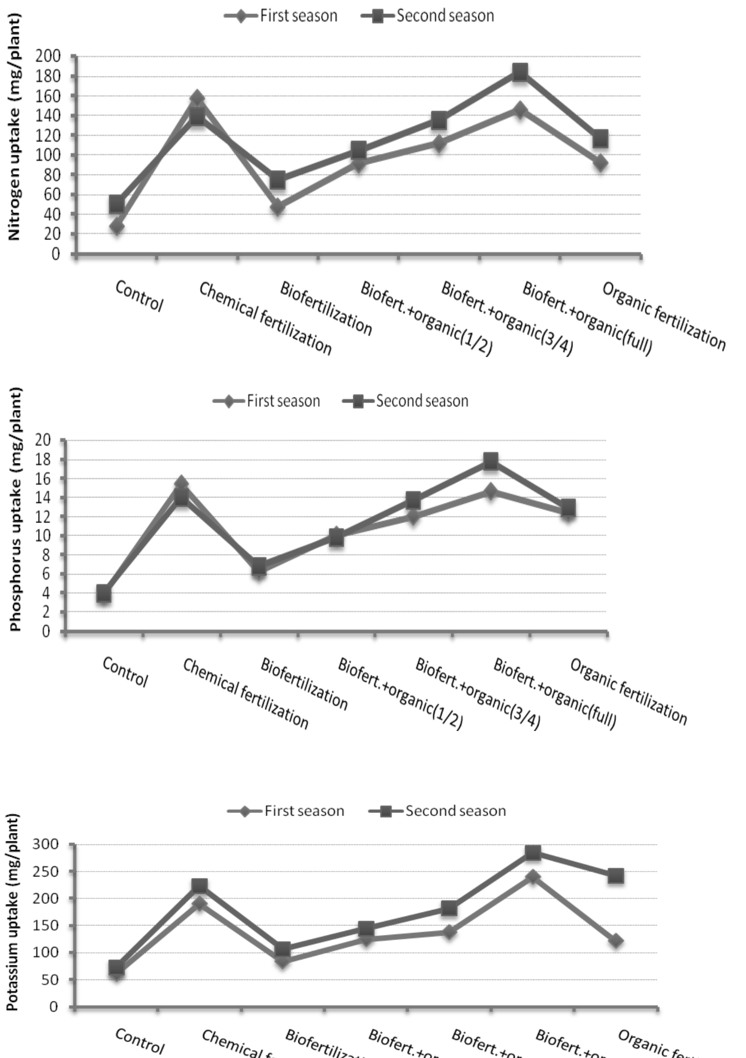


Fig 2. Effect of biofertilization and organic manuring on N, P and K uptake.

The enhancement of macronutrients uptake caused by organic manure may be due to the increase in available nitrogen, phosphorus and potassium.

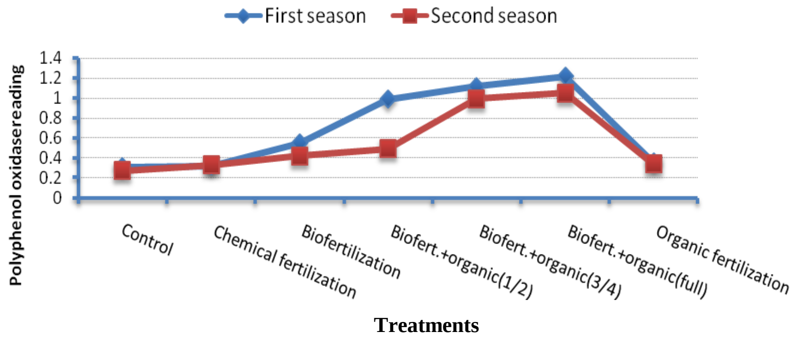
Macronutrients uptake by marjoram were higher in case of dual application with biofertilizers and compost than macronutrients uptakes obtained in either biofertilizers or compost. Increase in macronutrients uptake may be due to increase in macronutrients availability in soils treated with biofertilizers and organic manure. These results agree with those obtained by Rashed (2002) who reported that biofertilizers combined with organic manure increased the content of nitrogen, phosphorus and potassium uptakes by plants.

In addition, increase in macro-nutrients uptake may be due to effect of compost and microorganisms on root surface area per unit of soil volume and water-use efficiency (El-Ghadban *et al*., 2002), which directly affects the physiological processes and nutrients absorption.

Macro-nutrients content of marjoram shoots were higher in the 2nd season than in the 1st one. This difference between the two seasons may be due to the changes in the climatic conditions.

Effect of biofertilization and organic manuring on polyphenol oxidase and peroxidase activity in marjoram leaves

Data recorded in Fig. 3 clearly indicated that soil treated with either biofertilizers or organic manure had increased the activity of peroxidase and polyphenol oxidase.



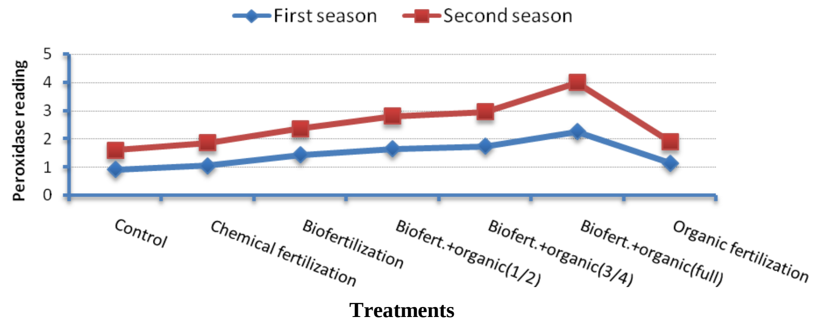


Fig. 3. Effect of biofertilization and organic manuring on polyphenol oxidase and peroxidase activity (as absorbance . g^{-1} fresh weight leaves of marjoram).

Chemical fertilization gave lower values of both peroxidase and polyphenol oxidase than soil treated with either biofertilizers or compost. This result could be attributed to the effect of microorganisms in compost or biofertilizers which have been demonstrated to induce systemic resistance including inhanced production of peroxidase and polyphenol oxidase. This result is in agreement with Gailite *et al*. (2005) who reported that the activity of both peroxidase and polyphenol oxidase increased in bean leaves after the treatment with plant growth promoting rhizobacteria (PGPR).

Marjoram inoculation with biofertilizer strains significantly increased the peroxidase and polyphenol oxidase activity compared to the control. These results are in harmony with those reported by Wei *et al*. (1991) and M'Piga *et al*. (1997) who demonstrated induction of systemic disease resistance in plant by PGBR. It may be due to their ability to increase peroxidase and polyphenol oxidase levels. Gamil (1995) proved that inoculation with *Paenibacillus polymyxa* increased peroxidase and polyphenol oxidase activity of squash leaves. Increasing the peroxidase and polyphenol oxidase activity in the PGPR treated plants may play either a direct or indirect role in the suppression of pathogen development in the host (Chen *et al*., 1998). Also, marjoram treatment with biofertilizers strains and organic manure gave greater records of polyphenol oxidase and peroxidase activities compared to treatments with either biofertilizer strains or organic manure. Marjoram inoculation with the biofertilizers combined with full dose of compost significantly increased the peroxidase and polyphenol oxidase activities.

Effect of biofertilization and organic manuring on oil percentage and oil yield of marjoram shoot

Data in Table 3 clearly showed that the mean values of essential oil of marjoram and oil yield per feddan were strongly affected by treatment with biofertilizers and/or amendment with compost compared with their corresponding controls. Increasing the quantity of organic manure in combination with biofertilizers led to an increase in essential oil percentage and oil yield of marjoram in both seasons. Similar trend of result was obtained by Edris *et al*. (2003).

Plants inoculation with biofertilizer strains resulted in significant increase of volatile oil percentage and oil yield per feddan compared to the control. In addition, inoculated plants with biofertilizer strains combined with full dose of compost resulted in 55.4 and 41.13 % increase in oil yield over plants with full doses of compost without inoculation in first and second season, respectively.

The highest values of essential oil percentage were obtained with chemical fertilization treatment although there was no significant difference in oil yield results between chemical fertilizer and biofertilizer with full dose of organic manure.

TABLE 3. Effect of biofertilization and/or organic manuring on oil percentage and oil yield of marjoram.

Treatments	Oil percentage		Oil yield (L / fed.)	
	First season	Second season	First season	Second season
Control*	0.70 [f]	0.90 [g]	4.23 [e]	5.75 [f]
Chemical fertilization	1.50 [a]	1.60 [a]	21.47 [a]	23.32 [a]
Biofertilization**	1.04 [e]	1.30 [f]	7.39 [d]	10.07 [e]
Biofert. + compost (1/2 dose)	1.20 [d]	1.39 [e]	11.62 [c]	13.96 [d]
Biofert.+ compost (3/4 dose)	1.30 [c]	1.44 [c]	14.60 [b]	18.40 [b]
Biofert.+ compost (full dose)	1.40 [b]	1.50 [b]	20.53 [a]	22.92 [a]
Full dose of compost	1.30 [c]	1.40 [d]	13.21 [b]	16.24 [c]

* Control : Non-fertilized and non-inoculated.
** Biofertilizers content: *P. polymyxa* H1+*B. megaterium* var. *phosphaticum* + *B. circulans*

From obtained results, it could be concluded that application of biofertilizers in combination with compost increased essential oil percentage and oil yield per feddan. Since, there isn't significant difference between this treatment and chemical fertilization one. The increase in oil yield might be due to either increase in vegetative growth or changes in leaf oil gland population and monoterpenes biosynthesis.

Effect of biofertilization and organic manuring on essential oil composition of marjoram

Fourteen compounds accounting for more than 97 % of the total volatiles in most marjoram shoot were detected and identified. The oil composition as affected by biofertilization and organic manuring. Summary of the predominant compounds in marjoram oil are presented in Table 4 as measured by gas mass chromatography (Fig. 4 a-g). Obtained data revealed that dual application of compost and biofertilization recorded the highest percentage of terpinen-4-ol (the major compound in marjoram oil), y-, α-terpinene, α-pinene, myrcene, sabinene, α-terpineol, (+)-2-carene and linalool accompanied by a decrease in the proportions of phellandrene and limonene compared to either compost or biofertilization treatments individually.

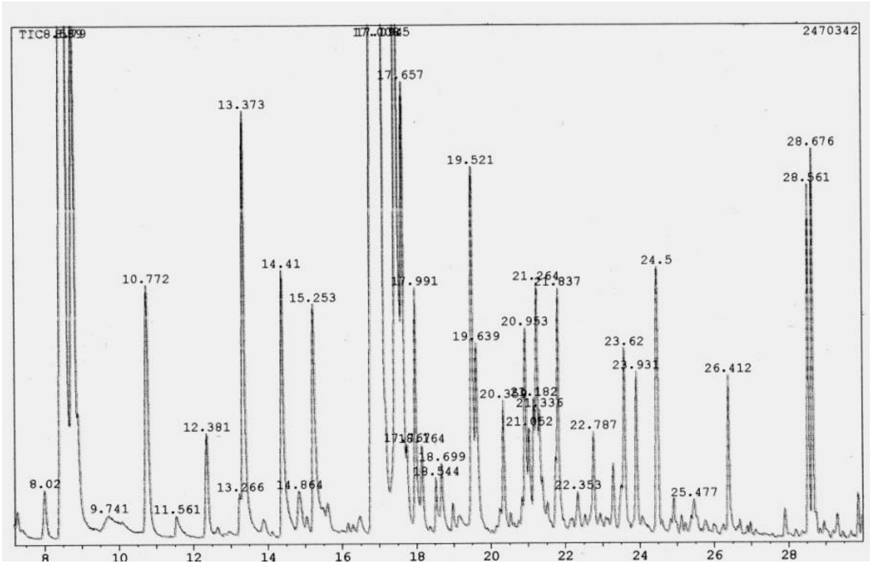
Biofertilization treatment produced the highest percentage of caryophyllene but the lowest percent of sabinene.

TABLE 4. Effect of biofertilization and/or organic manuring on composition of essential oil of marjoram.

Treatments / Oil components (%)	Retention time (min)	Control	Chemical fertilization	Biofertilization	Biofert. + compost ($^1/_2$ dose)	Biofert.+ compost ($^3/_4$ dose)	Biofert.+ compost (full dose)	Full dose of compost
Myrcene	7.69	1.29	0.7	1.4	1.45	1.5	1.56	1.29
(+)-2-carene	8.02	0.22	0.58	-	0.7	-	0.55	-
α-terpinene	8.57	22.97	20.91	23.41	19.48	23.53	24.08	17.28
Limonene	8.79	3.57	3.88	2.58	3.27	2.95	2.23	5.79
Linalool	13.37	1.72	1.37	1.75	1.05	1.56	2.74	0.81
p-cymene	16.13	0.8	0.62	0.68	0.7	0.49	0.49	0.73
Terpinen-4-ol	17.00	44.55	46.65	45.57	44.07	45.37	47.66	47.28
α-terpineol	17.65	7.51	7.76	7.52	6.72	7.64	8.18	6.7
Phellandrene	19.3	0.29	0.31	0.64	0.19	0.59	0.27	0.71
Sabinene	20.19	1.18	1.43	0.61	1.33	0.81	1.48	1.02
α-pinene	21.9	0.58	0.75	0.52	0.48	0.9	1.08	0.68
Camphene	23.9	0.33	0.34	0.3	0.19	0.4	0.28	-
Caryophyllene	24.5	0.94	0.72	1.35	0.53	1.1	0.34	1.33
y-terpinene	28.6	4.85	5.74	5.2	4.64	5.46	6.03	3.63
Unidentified	-	9.2	8.24	8.47	15.2	7.7	2.83	12.75

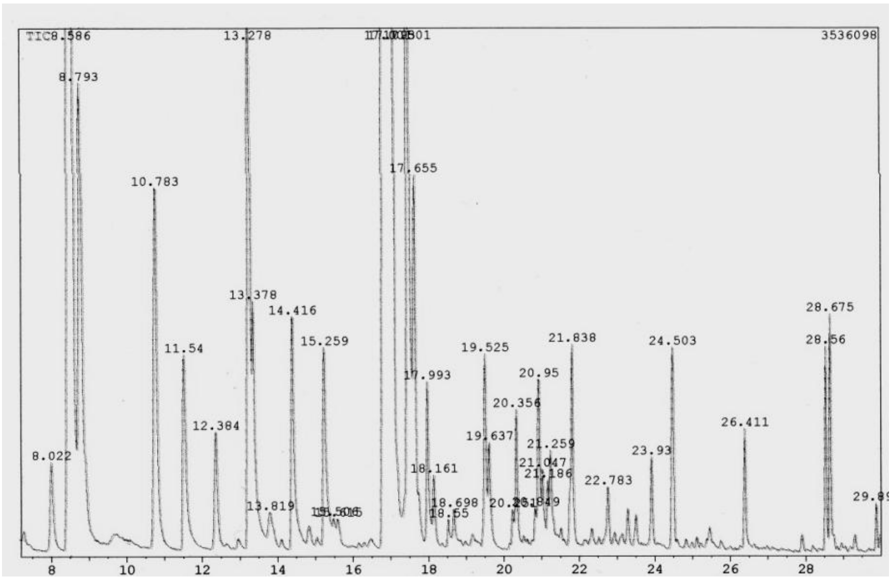
Moreover, chemical fertilization markedly increased the proportion of camphene and (+)-2-carene compared to other treatments. Edris *et al.* (2003) found that the relative percentage of certain constituents of marjoram essential oil were affected by fertilization type and level. Also, obtained data showed that (+)-2-carene compound was absent in the treatments of biofertilization, biofertilization + 3/4 dose and full dose of compost treatment. On the contrary, compost treatment recorded the highest percentage of phellandrene and limonene, whereas, camphene disappeared.

The major compound in marjoram oil is terpinen-4-ol is the major compound in marjoram oil followed by α-terpinene, α-terpineol, y-terpinene, limonene, linalool, myrcene, sabinene, caryophyllene, α-pinene, (+)-2-carene and camphene.



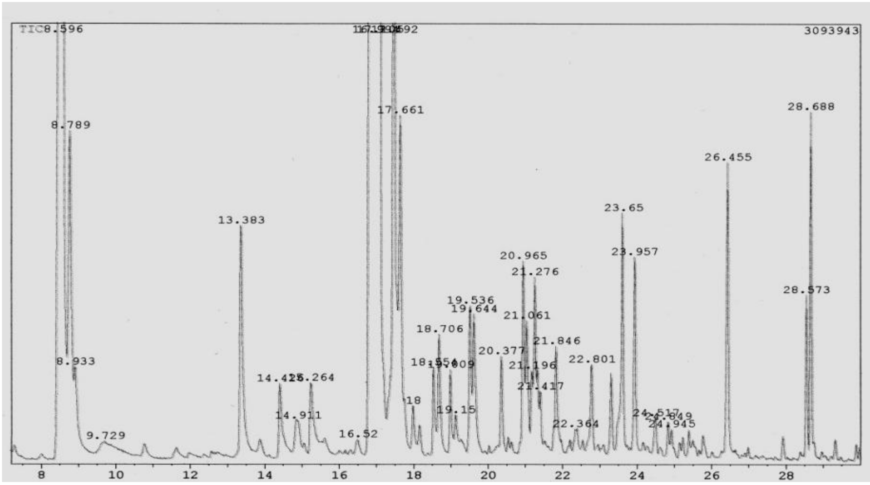
Retention time (min)

Fig 4-a. Gas liquid chromatography spectra of the essential oil components in marjoram grown under control treatment.



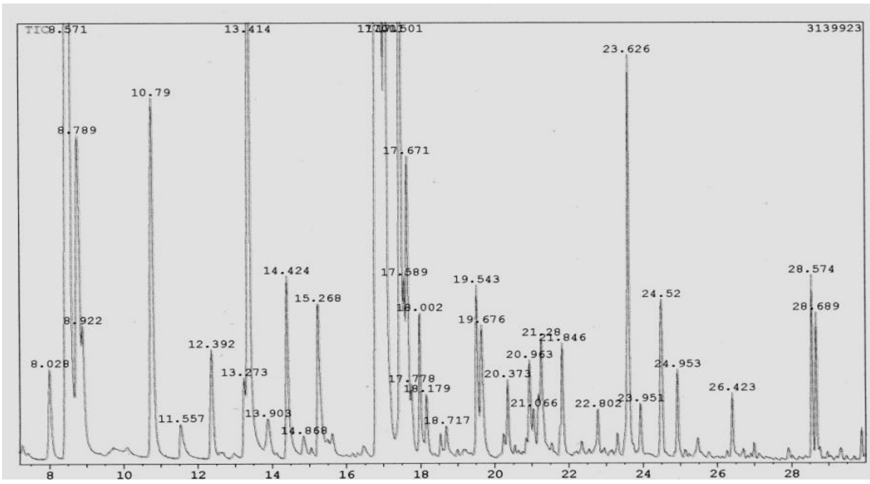
Retention time (min)

Fig 4-b. Gas liquid chromatography spectra of the essential oil components in marjoram grown under chemical fertilization treatment.



Retention time (min)

Fig 4-c. Gas liquid chromatography spectra of the essential oil components in marjoram grown under biofertilization treatment.



Retention time (min)

Fig 4-d. Gas liquid chromatography spectra of the essential oil components in marjoram grown under biofertilization+compost (1/2 dose) treatment.

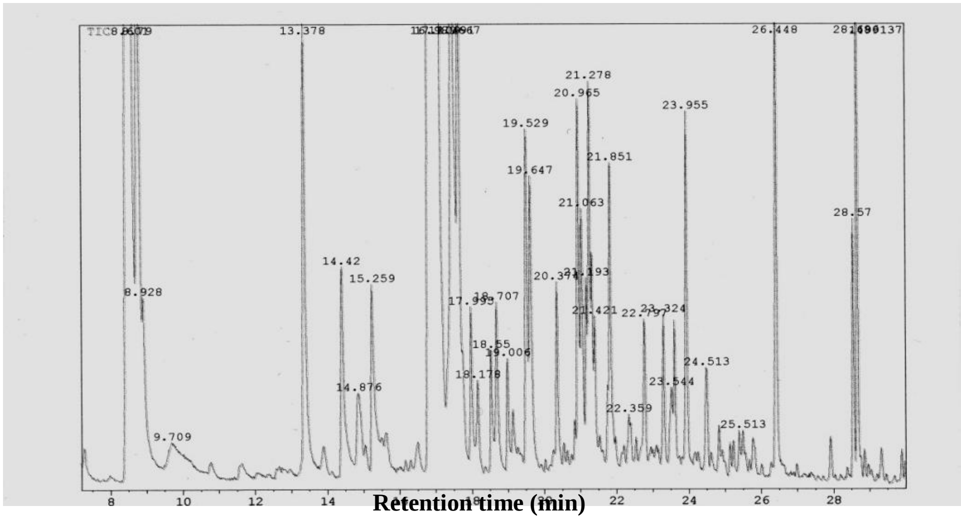


Fig 4-e. Gas liquid chromatography spectra of the essential oil components in marjoram grown under biofertilization+compost (3/4 dose) treatment.

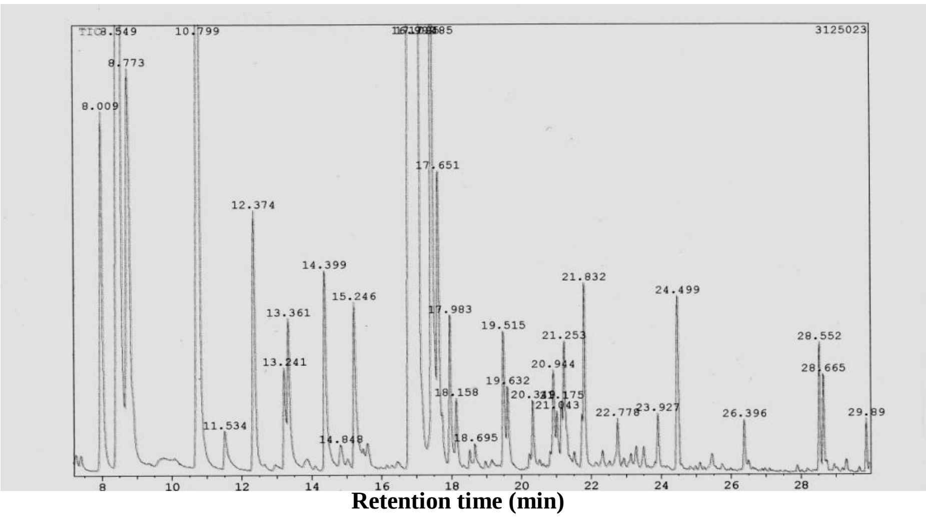
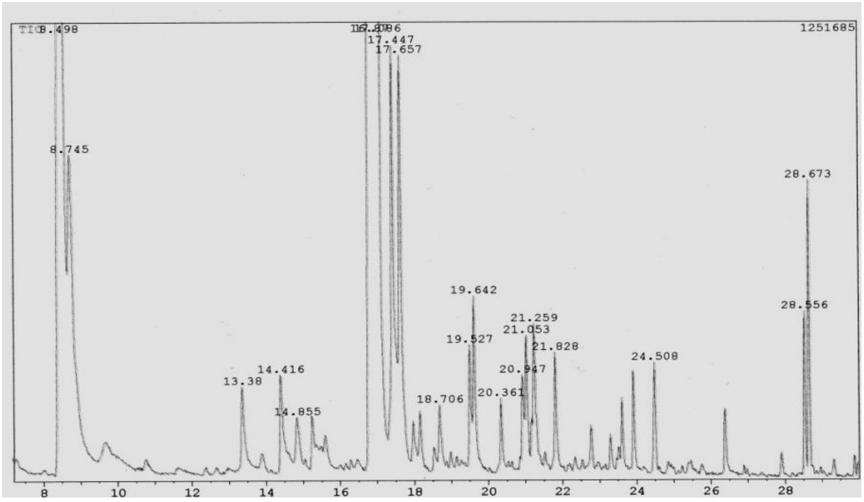


Fig 4-f. Gas liquid chromatography spectra of the essential oil components in marjoram grown under biofertilization+compost (full dose) treatment.



Retention time (min.)

Fig 4-g. Gas liquid chromatography spectra of the essential oil components in marjoram grown under full dose of compost treatment.

Conclusion and Recommendation

In view of the obtained results, it can be concluded that the dual application of biofertilization and organic manuring had great effect on dehydrogenase, peroxidase, polyphenol oxidase, macronutrients uptake, essential oil percentage and oil yield/ fed of marjoram. According to the obtained results, it can be recommended that combination of biofertilization and organic manuring together can substitute chemical fertilization in marjoram farming.

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تأثير التسميد الحيوى والعضوى على نشاط بعض الإنزيمات ومحتوى نبات البردقوش من العناصر المغذية والزيت العطرى

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أجريت تجربتان حقليتان فى محافظة المنوفية فى أحدى المزارع العضوية خلال موسمي ٢٠٠٦/٢٠٠٥ و٢٠٠٧/٢٠٠٦ لدراسة تأثير التفاعل بين التسميد الحيوي بمخلوط السلالات *Paenibacillus polymyxa* H1, *Bacillus megaterium var. phosphaticum* and *Bacillus circulans* والتسميد العضوي في وجود فطر *Trichoderma harzianum* على نشاط بعض الإنزيمات ومحتوى نبات البردقوش من العناصر المغذية والزيت العطر ي و لقد أوضحت النتائج ما يلى:

أظهرت النتائج ارتفاع في نشاط إنزيم الديهيدروجينيز عند تلقيح التربة بمخلوط سلالات التسميد الحيوي مقارنة بمعاملة التربة بالسماد العضوي.

أوضحت النتائج أيضا أن تلقيح نباتات البردقوش بسلالات السماد الحيوى أدى إلى زيادة نشاط إنزيمى peroxidase و polyphenol oxidase مقارنة بالنباتات الغير ملقحه. كما أدى التلقيح بالسماد الحيوى مع إضافة السماد العضوى إلى زيادة معنوية فى نشاط إنزيمى peroxidase و polyphenol oxidase داخل النبات مقارنة باستخدام كلٍ منهما على حدة.

أيضا أوضحت النتائج زيادة محتوى النباتات من النيتروجين والفوسفور والبوتاسيوم الممتص عند التلقيح بمخلوط سلالات السماد الحيوى وإضافة السماد العضوى مقارنة باستخدام كلٍ منهما على حدة. كما أظهرت النتائج أن أعلى تركيز من هذه المغذيات ظهر عند التلقيح بمخلوط سلالات السماد الحيوى واستخدام الجرعة الكاملة من السماد العضوى الصناعى.

وبخصوص محصول الزيت أوضحت النتائج أن النسبة المئوية لمحصول الزيت قد زادت معنويا عند التلقيح بمخلوط سلالات السماد الحيوى مع إضافة السماد العضوى الصناعى مقارنة ببقية المعاملات.

ومن الجدير بالذكر أن أعلى نسبة مئوية للزيت قد ظهرت عند تسميد التربة بالسماد الكيماوى في حين أوضحت النتائج أنه لا يوجد فرقا معنويا لمحصول الزيت بين النباتات المنتجة بالسماد الكيماوي (الزراعة التقليدية) والنباتات المنتجة تحت ظروف الزراعة العضوية. من خلال تحليل الزيت باستخدام أجهزة التحليل الكروماتوجرافى فقد ظهر ١٤ مركب تمثل ٩٧٪ من المواد الفعالة بالزيت العطرى. وقد أوضحت النتائج أن مكونات الزيت تأثرت معنويا بالتسميد الحيوى والعضوى، حيث أدى إنتاج البردقوش باستخدام التسميد الحيوى والعضوى إلى ارتفاع محتوى الزيت من terpinen-4-ol وهو المركب الرئيسى فى تركيب الزيت العطرى.