Interaction effect between biofertilization and organic manuring on some enzymes activity macronutrients and essential oil content of marjoram

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ABSTRACT

Field experiment was carried out at an organic farm during 2005- 2006 and 2006-2007 seasons. This experiment was carried out to study the efficiency of biofertilization and compost amendment in presence of *Trichoderma harzianum* on dehydrogenase, macronutrients uptake, polyphenol oxidase and peroxidase activity, oil yield and oil composition of marjoram. Obtained data showed that higher activity of dehydrogenase in rhizosphere and polyphenol oxidase & peroxidase were observed with dual application of biofertilization and organic manuring in presence of *T. harzianum* rather than each one individually. Similar trend of results was observed with N, P and K uptake since the highest records appeared with dual application of biofertilization and organic manuring. There isn't significant difference of oil yield between the chemical fertilization treatment and biofertilizers plus compost full dose treatment.

key words: Marjoram, biofertilization, organic manuring, *T. harzianum*, dehydrogenase, macronutrients uptake, marjoram essential oil yield and oil composition.

INTRODUCTION

Marjoram (*Majorana hortensis*) is aromatic herb and was known to the ancient Egyptians. It has been used not only to flavour food but also as a miraculous herb with the power to heal practically various diseases. Their essential oils have been known since antiquity to possess biological activity, notably antibacterial, antifungal as well as antioxidant properties (**Tiziana and Dorman, 1998**).

To overcome the ecological problems resulting from the loss of plant nutrients and to increase crop yield, microorganisms that allow more efficient nutrient use or increase nutrient availability can provide sustainable solutions for present and future agricultural practices. It is well known that the biofertilizers contain a variety of beneficial microorganisms which accelerate and improve plant growth and protect plants from pests and diseases. (**Abou-Aly *et al*, 2006**).

Marjoram inoculation with biofertilizer strains significantly increased the peroxidase and polyphenol oxidase compared to uninoculated one. The highest records of peroxidase and polyphenol oxidase were observed with the treatment of biofertilization combined with full dose of compost.

El-Ghadban *et al* (2002) mentioned that marjoram treatment with compost and biofertilizer led to an increase in macro-nutrients. These increases might be related to the positive effect of compost and microorganisms.

Application of biofertilizers (*Azotobacter chroococcum, Azospirillum lipoferum, Paenibacillus polymyxa* and *Bacillus megaterium*) increased nitrogen, phosphorus and potassium content in marjoram herb **(Mahfouz, 2003)**

Gailite *et al* (2005) reported that the content of both peroxidase and polyphenol oxidase increased in bean leaves after the treatment with plant growth promoting rhizobacteria.

Gharib *et al* (2008) reported that the using of combined treatment from biofertilizers and compost gave better results oil constituents of majoram than those obtained from either N_2 fixers (*Azospirillum brasiliense, Azotobacter chroococcum* and *P. polymyxa*), *B. circulans* or compost each one alone.

Leithy *et al* (2006) found that the essential oil components of rosemary were increased with the application of biofertilizers. **Azizi *et al,* (2008)** on chamomile reported that essential oil yield was increased with the increasing of compost dose.

Therefore, this research was carried out to study the various treatments of organic farming in the form of combination between organic manure, biofertilizers and biocontrol agent and their effects on some enzymes activity, marjoram oil yield and oil constituents.

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., Ministery of Agriculture.

Experimental soil.

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

Table1. Mechanical and chemical analyses 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**
E.C. (ds/m)			**2.02**
Organic mater %			**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**
		$CO_3^{=}$	**0**
		Cl^{-}	**11.2**
		$SO_4^{=}$	**4.6**
Total and available macronutrients	**N**	**total**	**1730**
		available	**53.64**
	P	**total**	**561.2**
		available	**130.94**
	K	**total**	**3500**
		available	**1612.8**

Soil determinations were carried out according to the method described by **Page *et al* (1982)**.

The used organic manure.

The main characteristics of compost which obtained from organic farm in Minoufia Governorate, GHAREEB SONS FARMS, are given in Table (2).

Table 2. Chemical analysis of the used compost in this experiment.

Parameters	Unit	Value
pH	-	8.11
EC (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

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).

Trichoderma harzianum was added to all investigation treatments

Inocula preparation

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

Cultivation process.

Prior to cultivation, plant bed was prepared. Seeds of marjoram were sown in prepared seed beds on 15^{th} October, for both study seasons. After 50-60 days, seedlings 10-15 cm length 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 tow 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 ½ dose 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 each *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 minutes before transplanting. Sucrose solution (30%) was added as an adhesive agent prior to inoculation. The same prepared inocula were added to the soil three times throughout the growing seasons.

Determinations

- **Dehydrogenase activity (DHA)** was assayed in soil according to **Thalmann (1967)**.
- **Total nitrogen, total phosphorus and total potassium** were determined in shoots according to the method described by **A.O.A.C. (1980), A.PH.A.(1992) and Dewis** and **Freitas (1970)** respectively**,** after that the micro-nutrients uptake per plant was calculated by the following equation**:**
- **Determination of resistance enzyme activities in plants.**

Peroxidase and polyphenol oxidase was determined according to the method described by **Allam** and **Hollis (1972)** and **Matta** and **Dimond (1963)** respectively**.**

- Essential oil of air dried herb was extracted by water distillation for 3 hours and then dried over anhydrous sodium sulphate and determined according to **Guenther (1961).** The oil yield per plant and feddan were calculated.
- **Statistical analysis.**

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

RESULTS AND DISCUSSIONS

Interaction effect of biofertilization and organic manuring on dehydrogenase in soil.

Data presented in **Fig (1)** showed that DHA is widely varied among the studied treatments.

The lower records of DHA were observed in soil amended with chemical fertilizers than the treatment of soil treated with biofertilizers and/or compost. This result was in accordance with **Krishnakumar *et al* (2007)** who reported that the recommended NPK fertilizers have significantly lower values of DHA than organic manure.

Higher records of DHA were observed in inoculated treatments 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 accumulation of available nutrients and stimulate the microorganisms in rhizosphere.

Moreover, data showed that the inoculation with biofertilizer strains combined compost gave 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 mentioned that DHA was higher in organic manure treatments, indicating an increase in the microbial metabolism in soil as a result of mineralization of biodegradable carbon compounds.

Data revealed that the soil inoculation with biofertilizer strains and *T. harzianum* combined with full dose of compost gave the highest values of DHA. This result is logic and was expected and this may be not due only to the effect of inoculation on microbes' number in rhizosphere but also to the beneficial effect of compost on indigenous and introduced biofertilizer strains proliferation 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.

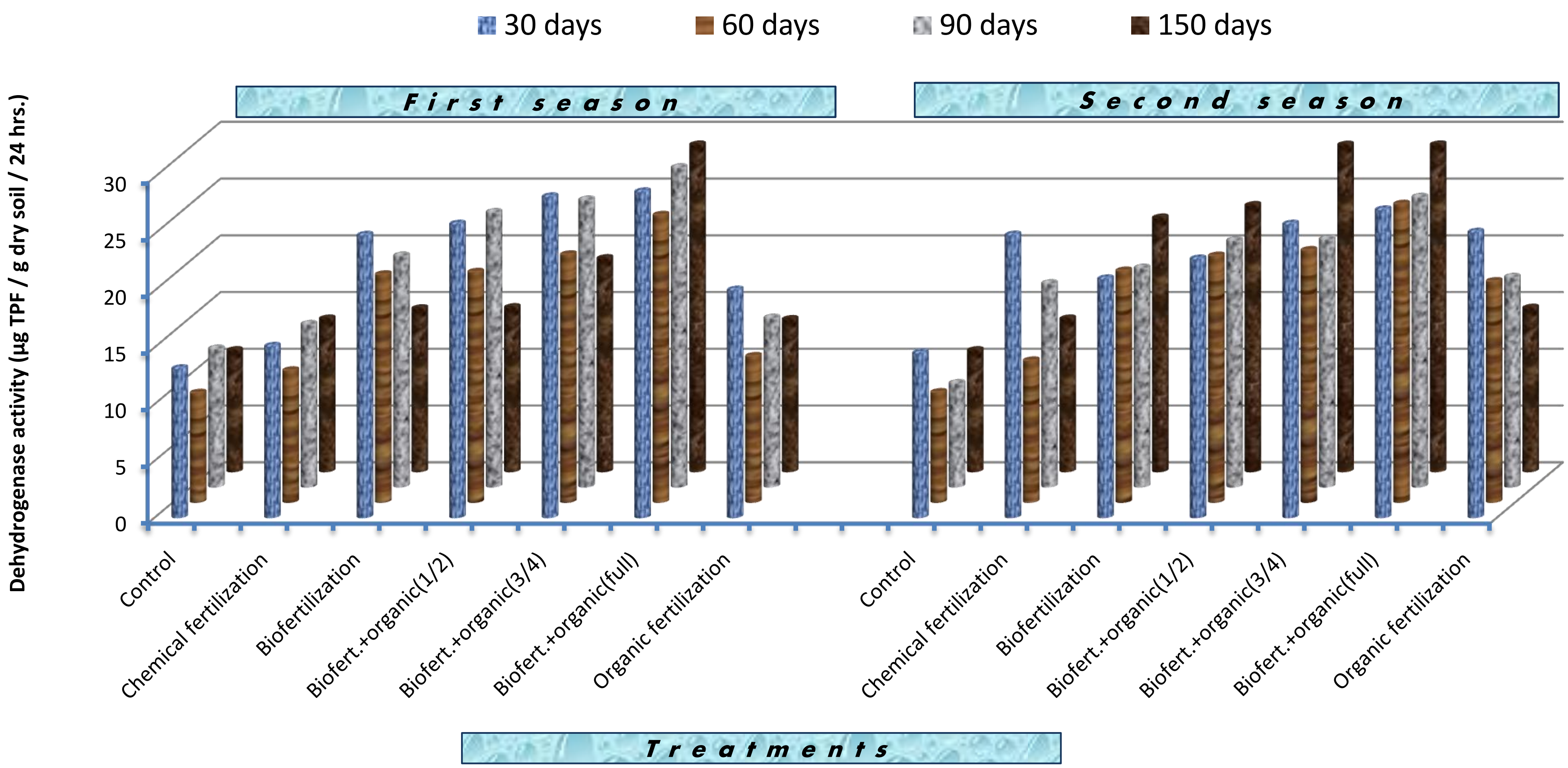


Fig 1. Interaction effect of biofertilization and organic manuring on dehydrogenase in soil cultivated with marjoram.

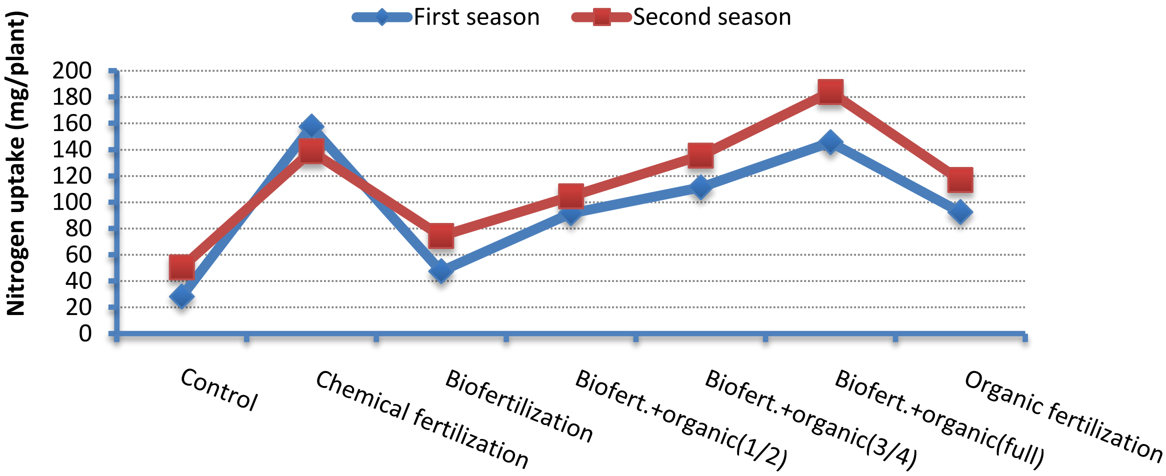
Interaction effect of biofertilization and organic manuring on N, P and K uptake.

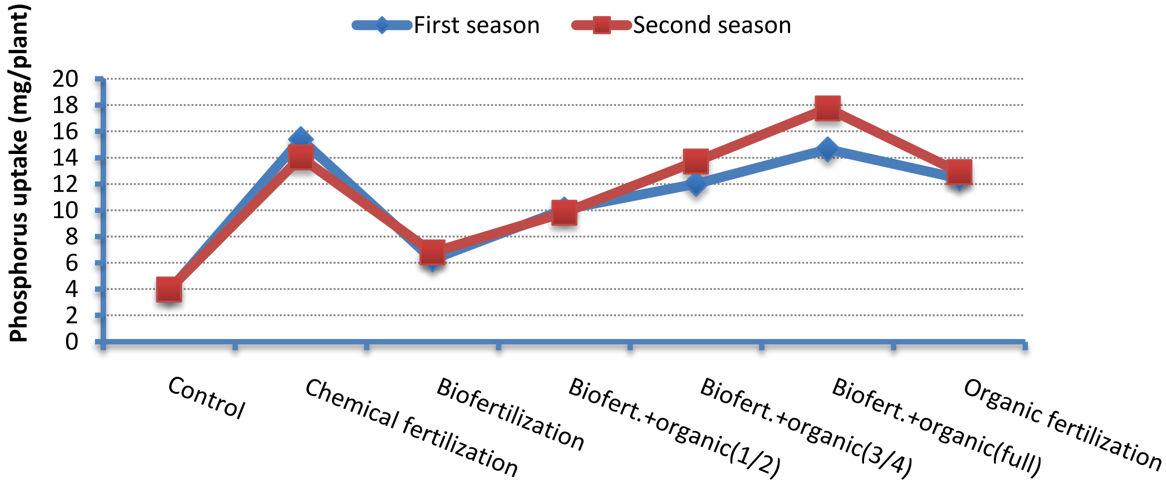
Obtained data (Fig, 2) showed that N,P and K uptake significantly increased in plants grown in soil amended with compost than soil inoculated with biofertilizer strains. The enhancement of macronutrients uptake caused by organic manure may be due to the increase of availability of nitrogen, phosphorus and potassium.

Macronutrients uptake by marjoram were higher in case of dual application with biofertilizers combined with compost than those recorded in either biofertilizers or compost each one solely. This result may be due to the beneficial effect of dual application on macronutrients availability and uptake by plants. These results confirm by those obtained by **Rashed (2002)** who reported that biofertilizers combined with organic manure increased the content of nitrogen, phosphorus and potassium.

El-Ghadban *et al* (2002) mentioned that both compost and biofertilizers led to an increase of macro-nutrients uptake. These increase might be related to the positive effect of compost and microorganisms in increasing the root surface area per unit of soil volume and water-use efficiency, which directly affects the physiological processes and nutrients absorption.

Inoculated plants with biofertilizers combined with full dose of compost gave the highest uptake of total nitrogen, phosphorus and potassium.





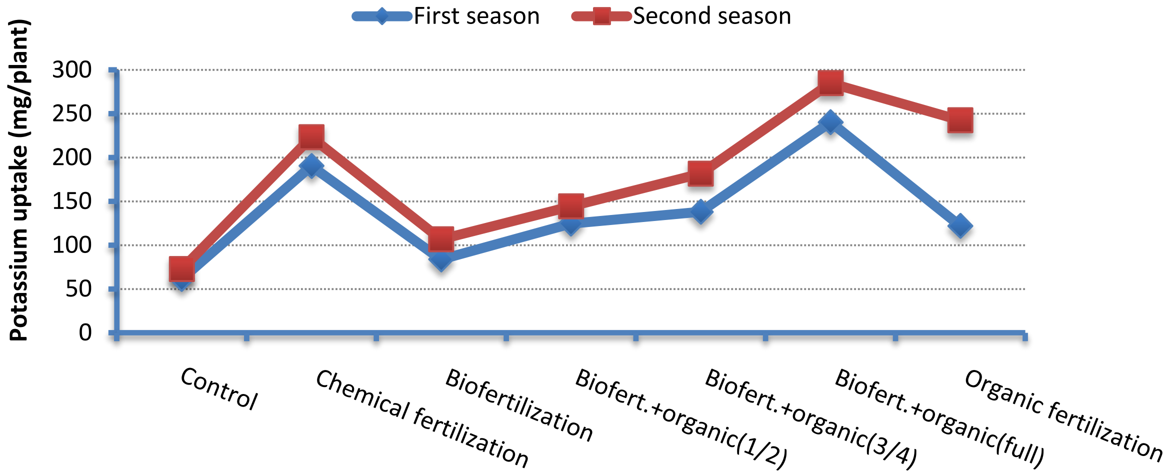


Fig 2. Interaction effect of biofertilization and organic manuring on N, P and K uptake.

Generally, macro-nutrients content in 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.

Interaction effect of biofertilization and organic manuring on polyphenol oxidase and peroxidase activity in marjoram.

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

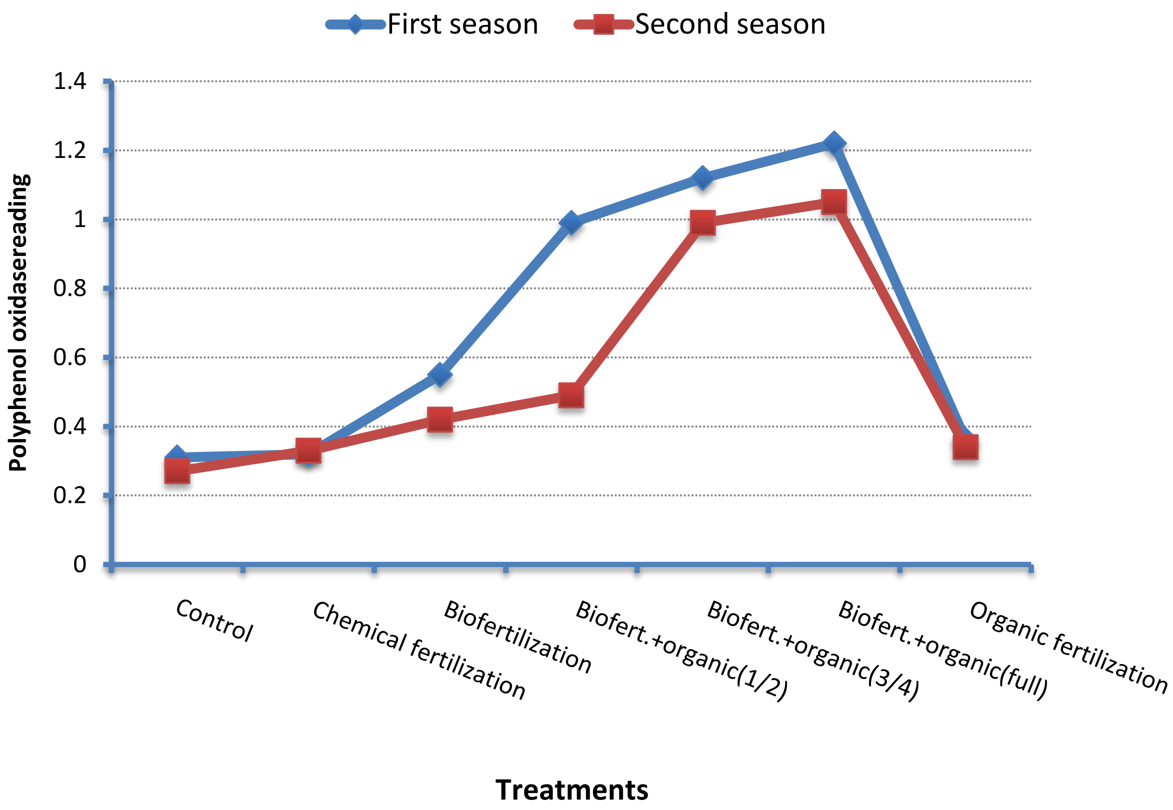
Chemical fertilization gave lower values of both peroxidase and polyphenol oxidase rather than soil treated with either biofertilizers or compost. This result could be attributed to the beneficial effect of microorganisms in compost or biofertilizers which have been demonstrated to induce systemic resistance including increase 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 un-inoculated ones. These results are in harmony with those stated by **Wei *et al* (1991)** and **M'Piga *et al* (1997)** who reported that PGPR has been demonstrated to induce systemic resistance to a variety of diseases. 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 inoculation with biofertilizers strains combined with compost gave higher records of polyphenol oxidase and peroxidase activity in comparison with marjoram inoculation with either biofertilizer strains or compost amendment individually.

Marjoram inoculation with the biofertilizers combined with full dose of compost significantly increased the peroxidase and polyphenol oxidase activity.



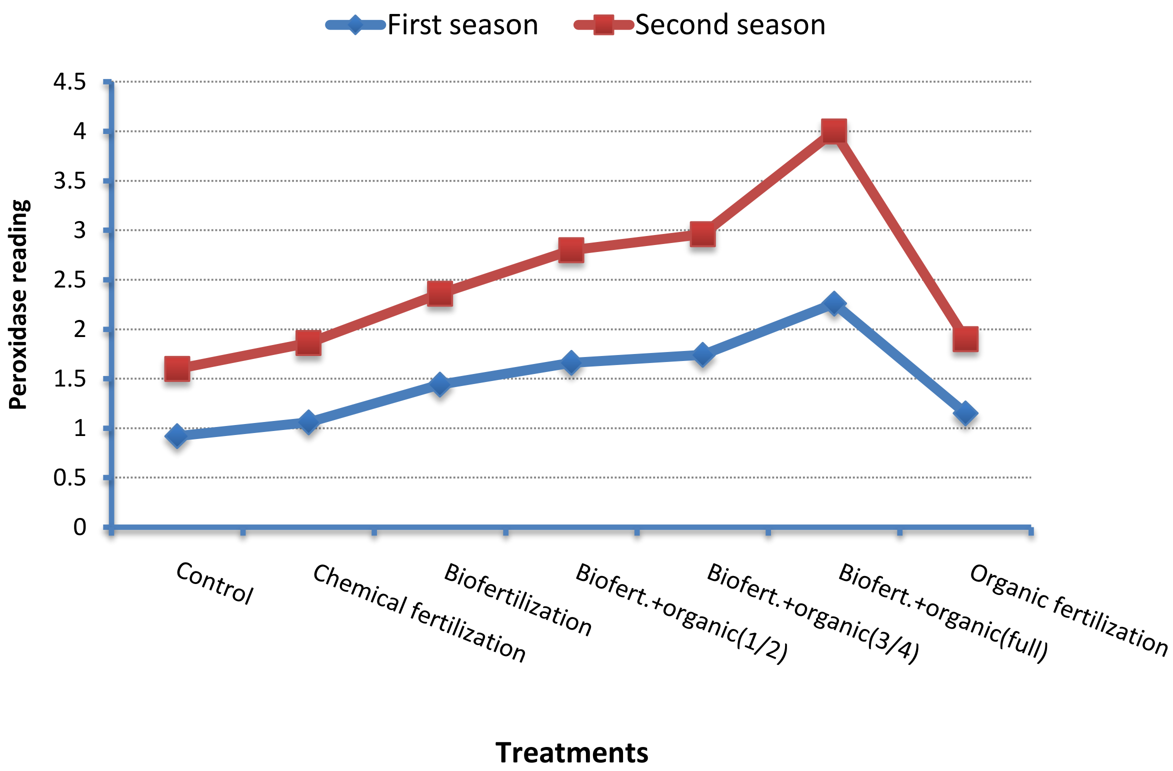


Fig 3. Interaction effect of biofertilization and organic manuring on polyphenol oxidase and peroxidase activity (as absorbance . g^{-1} fresh weight leaves of marjoram).

Interaction effect of biofertilization and organic manuring on oil percentage and oil yield of marjoram.

Data in **Table (3)** clearly showed that the mean values of essential oil of marjoram and oil yield per feddan were strongly affected by inoculation with biofertilizers and/or amendment with compost compared with their corresponding controls.

Increasing the rate 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**).

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*

Plants inoculation with biofertilizer strains caused significant increase of volatile oil percentage and oil yield per fedd. compared to un-inoculated one. 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 the fertilized plants with full doses of compost without inoculation in first and second season, respectively.

The highest values of essential oil percentage appeared with chemical fertilization, while the highest yield of the essential oil per feddan was observed in treatment of biofertilization combined with full dose of organic manuring or chemical fertilization treatment.

From obtained results, it could be concluded that applying 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.

Interaction effect of biofertilization and organic manuring on essential oil composition of marjoram.

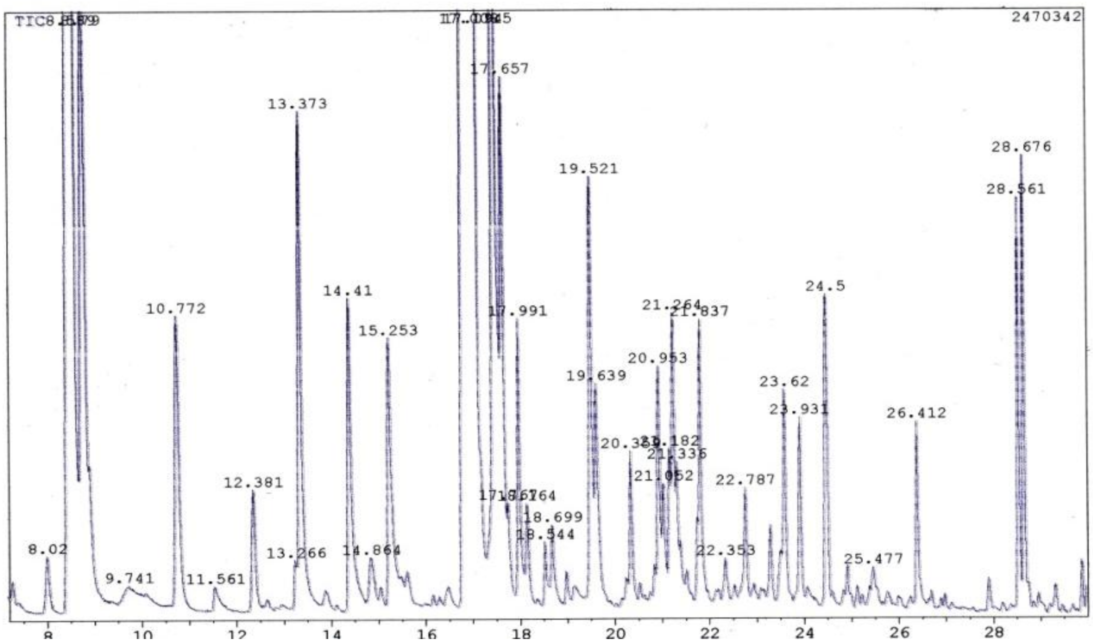
Fourteen compounds accounting for more than 97 % of the total volatiles in most marjoram samples were detected and identified. There are differences in oil composition as affected by biofertilization and organic manuring. The predominant compounds presented under all treatments were recorded in **Table (4).** 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.

Whereas, biofertilization treatment recorded the highest percentage of caryophyllene but the lowest percent of sabinene. 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 had disappeared in the treatments of biofertilization, biofertilization + 3/4 dose and full dose of compost. On the contrary , compost treatment recorded the highest percentage of phellandrene and limonene . whereas, camphene disappeared.

Generally, it can be noticed that 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.

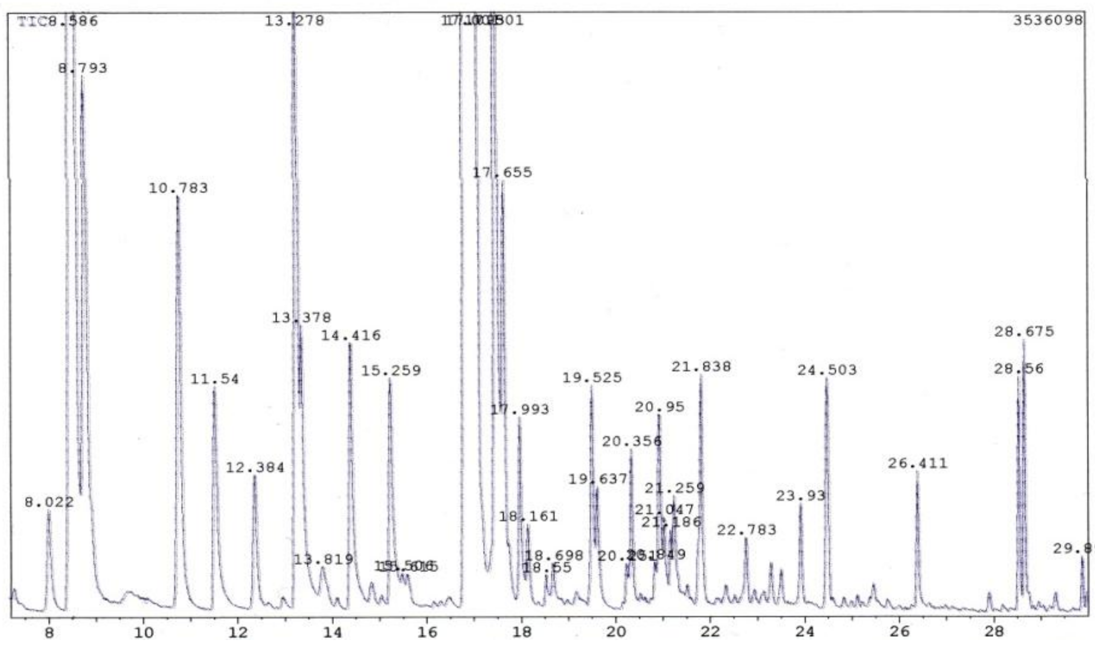
Table 4. Effect of biofertilization and/or organic manuring on composition of essential oil of marjoram.

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



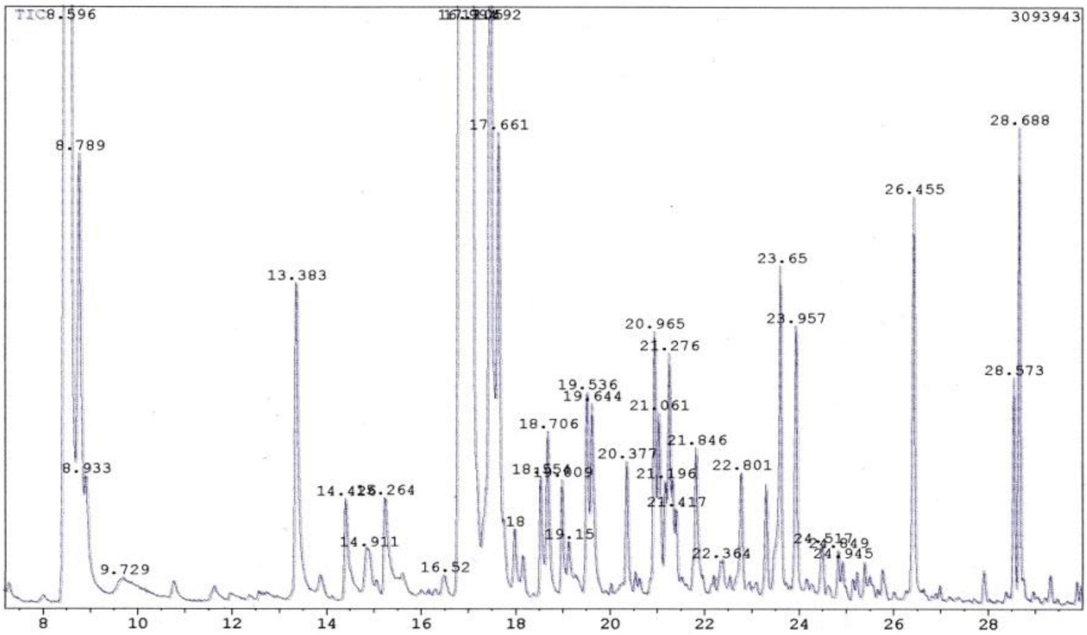
Retention time (minutes)

Fig 22a-1. Gas liquid chromatography spectra of the essential oil components in marjoram grown under control treatment.



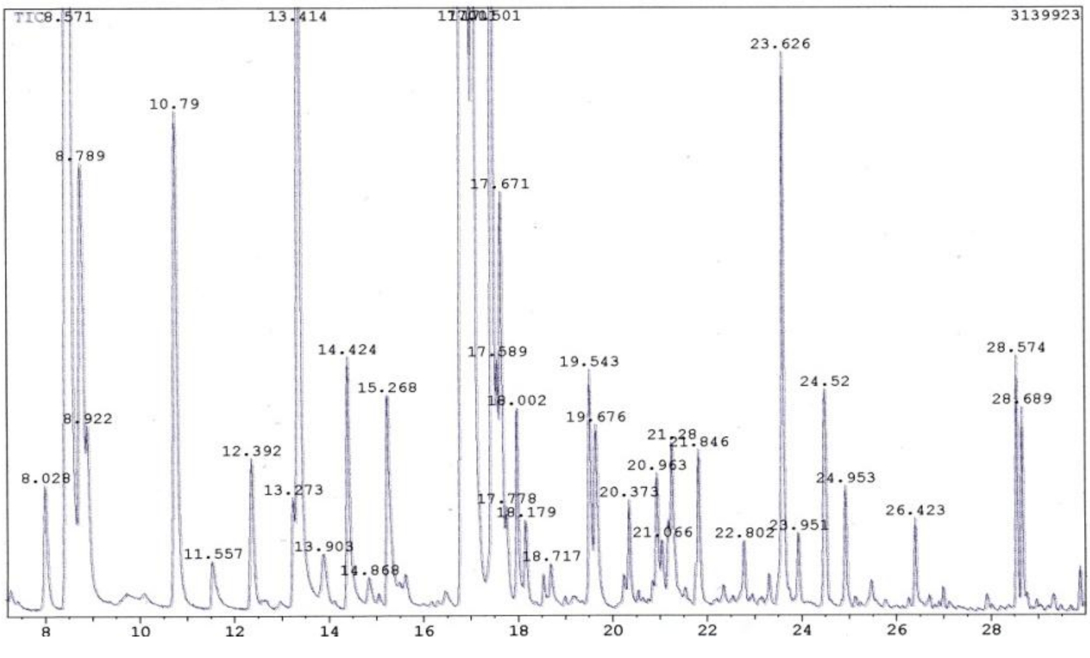
Retention time (minutes)

Fig 22a-2. Gas liquid chromatography spectra of the essential oil components in marjoram grown under chemical fertilization treatment.



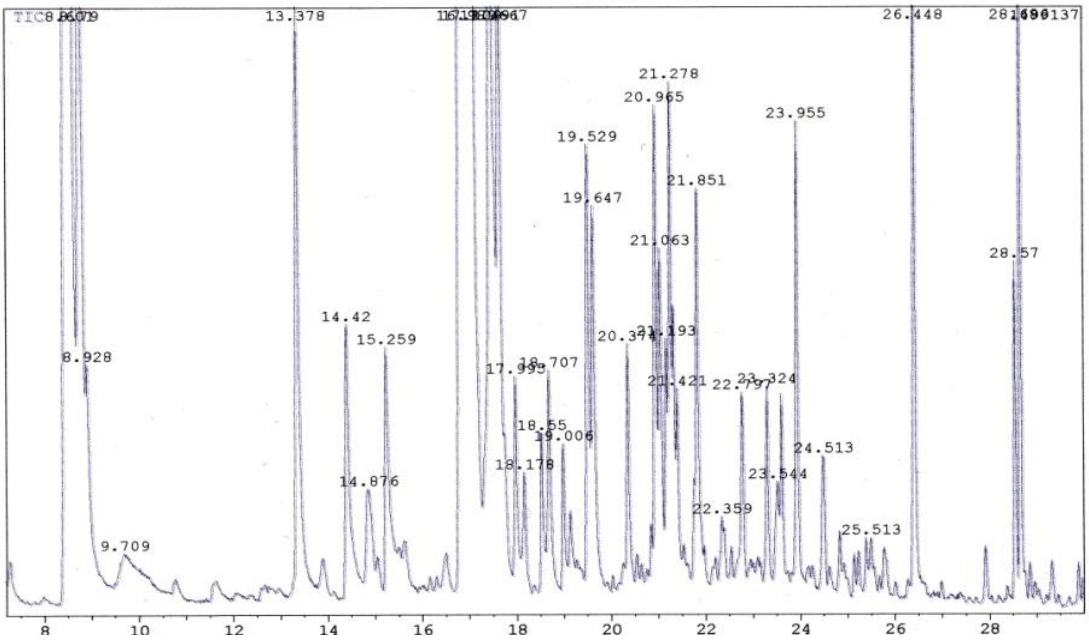
Retention time (minutes)

Fig 22a-3. Gas liquid chromatography spectra of the essential oil components in marjoram grown under biofertilization treatment.



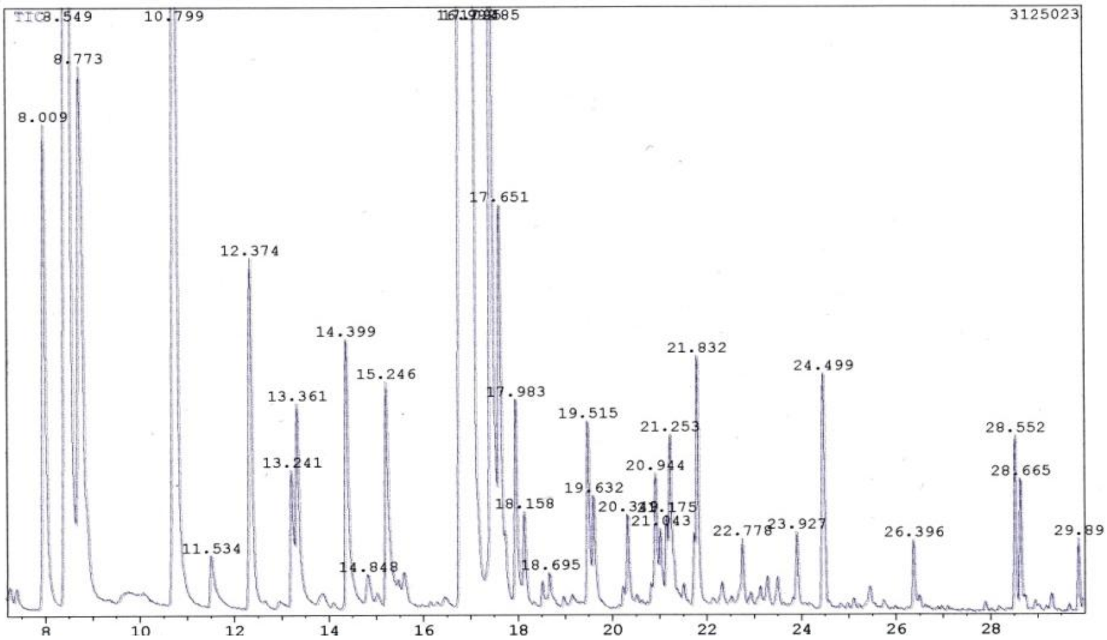
Retention time (minutes)

Fig 22a-4. Gas liquid chromatography spectra of the essential oil components in marjoram grown under biofertilization+compost (1/2 dose) treatment.



Retention time (minutes)

Fig 22a-5. Gas liquid chromatography spectra of the essential oil components in marjoram grown under biofertilization+compost (3/4 dose) treatment.



Retention time (minutes)

Fig 22a-6. Gas liquid chromatography spectra of the essential oil components in marjoram grown under biofertilization+compost (full dose) treatment.

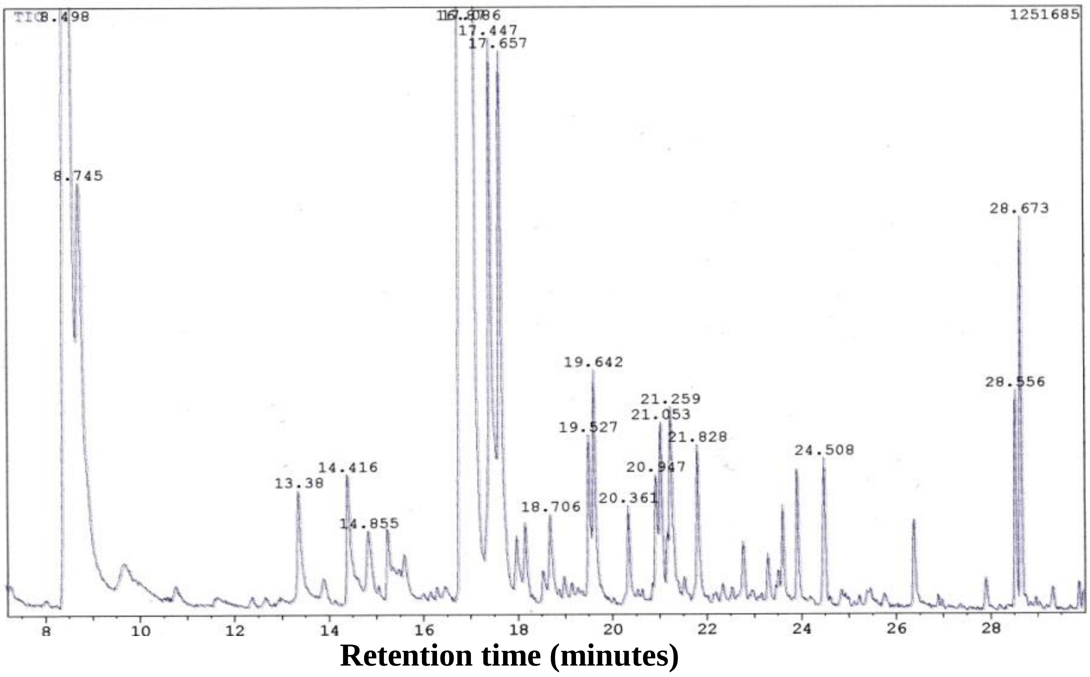


Fig 22a-7. Gas liquid chromatography spectra of the essential oil components in marjoram grown under full dose of compost treatment.

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 and essential oil percentage and oil yield/ fedd of marjoram. According to the obtained results, it can be recommended that the use of both biofertilization and organic manuring together can substitute chemical fertilization to obtain high productivity from marjoram. In addition, to obtain safety plant and reduce the environmental pollution

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تأثير التفاعل بين التسميد الحيوي والعضوي علي نشاط بعض الإنزيمات ومحتوي نبات البردقوش من العناصر المغذية والزيت العطري

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أجريت تجربتان حقليتان في محافظة المنوفية في أحد ي المزارع العضوية خلال موسمي ٢٠٠٥/٢٠٠٦ و٢٠٠٦/٢٠٠٧ 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 بعض نشاط الإنزيمات ومحتوى نبات البردقوش من العناصر المغذية و الزيت العطري ولقد أوضحت النتائج ما يلي:

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

وعند التلقيح بالسماد الحيوي مع إضافة السماد العضوي أدى ذلك إلى ارتفاع ملحوظ في نشاط إنزيم الديهيدروجينيز مقارنة بالمعاملة بكل منهما على حده.

أيضا أوضحت النتائج زيادة محتوي النباتات من النيتروجين والفوسفور والبوتاسيوم الممتص عند التلقيح بمخلوط سلالات السماد الحيوي وإضافة السماد العضوي مقارنة باستخدام كلٍ منهما على حدة . كما أظهرت النتائج أن أعلي تركيز من هذه المغذيات ظهر عند التلقيح بمخلوط سلالات السماد الحيوي واستخدام الجرعة الكاملة من السماد العضوي الصناعي.

أوضحت النتائج أيضاً أن تلقيح نباتات البردقوش بسلالات السماد الحيوي أدى إلى زيادة نشاط إنزيمي polyphenol oxidase &peroxidase مقارنة بالنباتات الغير ملقحه . كما أدي التلقيح بالسماد الحيوي مع إضافة السماد العضوي إلي زيادة معنوية في نشاط إنزيمي polyphenol oxidase & peroxidase داخل النبات مقارنة باستخدام كلٍ منهما على حدة.

وبخصوص محصول الزيت أوضحت النتائج أن النسبة المئوية لمحصول الزيت قد زادت معنويا عند التلقيح بمخلوط سلالات السماد الحيوي مع إضافة السماد العضوي الصناعي مقارنة ببقية المعاملات.

ومن الجدير بالذكر أن أعلي نسبة م ئوية للزيت قد ظهرت عند تسميد التربة بالسماد الكيماوي 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 الكروماتوجرافي فقد ظهر ١٤ مركب تمثل ٩٧% من المواد الفعالة بالزيت العطري. وقد أوضحت النتائج أن مكونات الزيت تأثرت معنويا بالتسميد الحيوي والعضوي، حيث أدي إنتاج البردقوش باستخدام التسميد الحيوي والعضوي إلي ارتفاع محتوي الزيت من terpinen-4-ol وهو المركب الرئيسي في تركيب الزيت العطري.