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Organic fertilization and composition of oregano essential oil

[Fertilización orgánica y composición de aceite esencial de organo]

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Abstract: *Origanum vulgare* L. better known as oregano or marjoram in Europe and Brazil is a herbaceous plant which determines long-range chemical and morphological diversity. The composition of essential oil of herbs like oregano, depends on different factors such as climate, geographical origin, harvest season, nutrition that can significantly affect the oil production and quality. The objective of this work was investigate the oregano oil production and composition in differents conditions of crops and seasons in Cerrado of Brazil. The experimental was a randomized block design (RBD) in a factorial 2 x 2 x 2 with five replications, two systems of planting (greenhouse and field), two types of fertilizers (mineral and organic) and two seasons (autumn and spring). The results of essential oil presented the same composition for all the treatments. However, the relative proportion of some chemical constituents was altered according to the planting in greenhouse or in field and organic or mineral fertilizer. Carvacrol, terpinene, ortho-cymene were the major constituents. The spring season and the field conditions favored better essential oil yield and carvacrol production.

Keywords: *Origanum vulgare* L., aromatic plant, composition, carvacrol

Resumen: *Origanum vulgare* L. mejor conocido como orégano o mejorana en Europa y Brasil es una planta herbácea que presenta una gran diversidad química y morfológica. La composición del aceite esencial de hierbas como el orégano, depende de diversos factores tales como el clima, la procedencia geográfica, época de la cosecha, la nutrición que pueden afectar significativamente la producción de aceites y su calidad. El objetivo de este trabajo fue investigar la producción de aceite de orégano y composición en diferentes condiciones de los cultivos y las estaciones en Cerrado de Brasil. La parte experimental consistió en bloques al azar (RBD) en un diseño factorial 2 x 2 x 2 con cinco repeticiones, dos sistemas de siembra (invernadero y de campo), dos tipos de fertilizantes (minerales y orgánicos) y dos estaciones (otoño y primavera). Los resultados del aceite esencial presentan la misma composición para todos los tratamientos. Sin embargo, la proporción relativa de algunos componentes químicos se modificó de acuerdo con la siembra en invernadero o en el campo y abono orgánico o mineral. Carvacrol, terpineno, orto-cimeno fueron los principales constituyentes. La temporada de primavera y las condiciones de campo favorecieron el rendimiento de aceite esencial y una mejor producción de carvacrol.

Palabras clave: *Origanum vulgare* L., planta aromática, composición, carvacrol

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INTRODUCTION

The *Origanum vulgare* L., better known as oregano or manjoram in Europe and Brazil, is an herbaceous plant and belongs to the family Lamiaceae, which also includes other herbs of popular use, such as lavender (*Lavandula angustifolia*), herb balm (*Melissa officinalis*), basil (*Ocimum basilicum*), rosemary (*Rosmarinus officinalis*), among others (Lorenzi & Matos, 2002).

The family Lamiaceae according to Souza (2005) has a big distribution in Brazil, there are 26 genera with 350 species. *Origanum vulgare* L. is native of Euro-siberian and Irano-siberian regions and has a long range of morphological diversity and chemistry (Iestwaart, 1980; Aligians *et al.*, 2001). In species *Origanum vulgare* L., agronomic research is most developed in Europe and North Africa (Padulosi, 1997). A high carvacrol content in essential oil is the key to the concept of the “oregano” spice and is a prerequisite determining a plant’s suitability for the preparation of this condiment (Fleisher & Sneer, 1982).

Essential oils are used to impart special flavor and odor to various food products and cosmetics. Also great is their use as analgesics, antiseptics, sedative, expectorant, stimulant and stomachic, among others. Scheffer (1992) reports that information about the behavior of condiments when subjected to agronomic techniques is restricted by these and other peculiarities, this species deserves more study and investment, because in spite of market acceptance, Brazil still imports some of what is consumed countries like Chile and other countries of the Mediterranean (Corrêa *et al.*, 2010).

Currently, Brazil has a prominent place in essential oil production, next to the India, China and Indonesia, which are considered the four major producers mundiais. Brazil's position is due to the citrus oil (juice industry). In the past, the country had featured as essential oil exporter of “pau-rosa” (*Aniba rosaeodora*), “sassafras” (*Ocotea odorifera*) and mint (*Mentha arvensis*), and about the latter two cases, passed the importer condition today (Bizzo *et al.*, 2009).

Brazil, due to its large territory, has characteristic peculiar to each climatic region, which affect positively or negatively on the development of native or introduced aromatics species, even if the conditions are similar to the native location. Therefore, before starting the cultivation on a commercial scale, it is necessary to know the behavior of the species with respect to the effects of climatic region of planting, cultivation and biotic factors that are responsible for plant development. The lack of technological mastery of all phases of development will likely lead to poor quality of the content of the biomass and chemical constituents of essential oil, as well as in income (Blank *et al.*, 2005). The objective of the work was to analyze the production of biomass, content, yield and composition of oregano essential oil under different systems and seasons of planting.

MATERIALS AND METHODS

The experiment was conducted in Gloria Research Farm (18°57’ S e 48°12’ W), part of Federal University of Uberlândia, Minas Gerais State of Brazil. According to the Köppen classification, the climate is characterized as Aw (megathermic), presenting dry winter and rainy summer. The soil in this area is characterized as Oxisoil, which has very advanced developments with a significant presence of latolization process, resulting in intense leaching of primary mineral constituents (Embrapa, 2006).

The field and greenhouse experiments were installed and conducted simultaneously. It was used commercial seeds of *Origanum vulgare* L., obtained from the company ISLA. This variety is wildly know like “oregano- type-carvacrol”. They were sown in trays of 200 cells on November 17, 2010 to be conducted during the autumn season of 2011 and May 10, 2011 to the spring season, respectivelly. They were kept in a nursery greenhouse and irrigated twice daily until the moment to be transplanted. This was done on January 23 and July 18, 2011, when the seedlings had six pairs of leaves. The experimental design was a randomized block design (RBD) in a 2 x 2 x 2 factorial two systems of planting (field and greenhouse), two types of fertilizer (chemical and organic) and two growing season (autumn and spring) with five replicates. The field and greenhouse plots had three rows of 3 m in length and the useful plot of each replicate were consisted of seven plants in the central line. The spacing has 40 cm between rows and 35 cm between plants. Irrigation was performed daily by the micro sprinkler system, except during the days that rainfall occurred. The weed control was made by hand hoeing at intervals of approximately 7 days from each other, until the moment of the harvest. There was no application of pesticides in installments throughout the period of area experimental.

About the fertilization, mineral and organic fertilizers were applied manually in total area of the plots, 30 days before transplantation and thus incorporated into the soil. Doses of organic and mineral fertilizers were based on recommendations for medicinal and aromatic plants, by Martins & Figueiredo (2009) and Ribeiro *et al.,* (1999), respectivelly. The organic fertilizer was composed by cattle manure, using dose of 4 kg m^{-2} with the following composition: N 2%, organic matter 71%, organic carbon 20%, P 0,5%, K 3%, Ca 2,34%, Mg 0,2%, B 33 mg kg^{-1}, Fe 1500 mg kg^{-1} and Na 95 mg kg^{-1}. The mineral fertilizer was compound by the granular formulated 04-14-08 of NPK, using dose 100 g m^{-2}.

The harvest occurs 115 days after the transplanting, practically. The harvest was done manually by cutting the plants at soil level, being done in the morning of May 19, 2011 (autumn season) and November 11, 2011 (spring season). There was no flowering of this plants. It were evaluated productivity of leaves, content, yield and essential oil chemical composition. The material was weighed on precision scales for measurement of fresh weight. The leaves were separated of the stems and were placed in paper bags and taked to the oven with forced air flow (65° C) to measure the dry mass. The water percentage was 75 to 80%. After that, the extraction of essential oil was submitted to quantify the content (%), yield and composition of the plants.

For extraction of fresh and dry matter were used 100g of leaves. The essential oil was obtained by hydrodistillation in a modified Clevenger apparatus. The process officially started when the first drops of essential oil condensed. The remaining extraction took two hours. The essential oil was stored in amber bottles and maintained in a freezer until the calculation of productivity and the chemical composition analysis. This analysis was accomplished at the Laboratory of Natural Products from IAC (Centre for Research and Development on Plant Genetic Resources). The qualitative analysis of the essential oil compounds was performed on a gas chromatography coupled to a mass spectrometer (GC-MS Shimadzu, QP5000), operating at an MS ionization voltage of 70 eV. The chromatography was equipped with a fused silica capillary column OV-5 (30 m x 0.25 mm x 0.25 µm), and helium was used as the carrier gas. The following chromatography conditions were used: injector at 240° C, detector at 230° C, gas flow 1.0 mL min^{-1}, split: 1/20, initial column temperature of 60 to 135° C at rate of 3° C min^{-1} then to 135 to 165° C at rate of 8° C min^{-1} then to 165 to 240° C at rate of 5° C min^{-1}, ending with a 5 min isothermal at 240° C, and injecting 1 µL of solution (1 mg of essential oil and 1 mL of ethyl acetate). The compounds were identified by the comparative analysis of the acquired mass spectra with those stored in the GC/MS database of the system (Nist 62. Lib), literature (McLafferty & Stauffer, 1989), and Kovats retention indices obtained by the co-injection of the samples and a mixture of *n*-alcanes (C_9H_{20}-$C_{25}H_{52}$, Sigma Aldrich, 99%) employing a column temperature program as follows: 60 to 240° C at a rate of 3° C min^{-1} (Adams, 2007).

The data obtained were compared by the variances analisis (ANOVA) and the means were compared by Tukey test ($p \leq 0.05$), with SISVAR software.

RESULTS AND DISCUSSION

Autumn season

The results presents that fresh biomass productivity (Table 1), the field conditions provided better production in two fertilizations. Oregano is a deriving of subtropical and tempered climates and needs much luminosity to better performance, not supporting extreme temperatures and high humidity of air (Verma *et al.,* 2010). When cultivated in field the temperature keeps more soft, the luminosity is bigger and lesser relative humidity, when compared with the conditions of the greenhouse.

Table 1
Fresh and dry mass (g plots^{-1}) of aereal part of *Origanum vulgare* L. in the autumn season

	Fresh mass			Dry mass		
	Grenhouse	**Field**	**Means**	**Grenhouse**	**Field**	**Means**
Mineral	232.58	595.97	414.28 a	55.96	146.95	101.45 a
Organic	240.20	574.80	407.50 a	67.93	140.14	104.03 a
Means	236.39 B	585.38 A		61.94 B	143.55 A	
DMS	101.25					23.36
CV%	25.29					23.34

D.M.S.: minimum difference significant; C.V. coefficient of variation; Means followed by distincts uppercase and lowercase letters in the row in the column do not differ by Tukey test at the 0.05 significance level.

Analyzing fertilization, Sodré (2007) also did not found significant differences between organic and mineral for production of fresh and dries biomass for *Melissa officinalis* and Chaves *et al.* (1998), with *Mentha villosa*, they did not obtain significant difference between mineral or organic fretilizers. However, Sales (2006) for *Hytis marrubioides* told that the organic fertilization provided a sharper accumulation of fresh biomass.

The organic fertilization using residue of another activity, like production of milk, it is economically more viable than mineral fertilizer. Moreover, it propitiates an improvement in the physical and biological conditions of the soil, increasing the capacity of exchange of cations and the text of organic substance, and can proporse the slow release of nutrients for the cultures. Differents this, the mineral fertilizer can cause acidification of soil, quickly provides nutrients to the soil and promote the leaching, which in some cases increases the cost of production in subsequent crops, requiring more application. Because of the soil features of Brazil that are highly weathered, the use of organic fertilizers can bring major benefits over mineral fertilizers.

The essential oil content (Table 2) also did not have significant interaction between fertilization and system of planting for fresh and dry biomass of oregano, but the field conditions presents better results. Botrel *et al.* (2010), analyzing the variations in contents of *Hyptis marrubioides* oil observed that plants cultivated in field had presented greater content of oil (0.13%), being on average three times bigger that in greenhouse (0.04%). Studies with subspecies of *Origanum vulgare* spp *vulagre*, spp *hirtum* and spp *viridulum*, distributed at Greece, had disclosed essential oil contents of dry biomass enter 0.1% up to 8%, values not registered for none another species of the Lamiaceae. The variation of the values is referring to the areas of soft temperature (20-25º C) with precipitations of 30 to 50 mm until more cold regions (14-18º C) with precipitation of 5 to 10 mm, respectively (Kokkini *et al.*, 1994). As the Mediterranean region is the center of origin of the species, this discloses that soft environmental conditions can guarantee better oil production.

The yield of essential oil not presented significant interaction (Table 3), but the oil production in the field was considerably bigger that the greenhouse. The yield results depends of the values of oil contents, so the differences founded had been influenced by the biomass production. In the field, the biomass production was bigger, therefore, the oil yield also bigger had to the increase of biomass for area. Similar results had been found by Nalepa & Carvalho (2007) with camomila (*Matricaria chamomila*), the increases in the essential oil productivity occurs in virtue of biomass production.

Table 2
Essential oil content of fresh and dry mass (%) of *Origanum vulgare* L. in the autumn season

	E.O. Content fresh mass			E.O. Content dry mass		
	Grenhouse	**Field**	**Means**	**Grenhouse**	**Field**	**Means**
Mineral	0.2284	0.3428	0.2855a	1.0570	1.1242	1.09062a
Organic	0.2642	0.2924	0.2783a	0.8608	1.2416	1.0512b
Means	0.2462B	0.3176A		0.9589B	1.1829A	
DMS	0.0617					0.2490
CV%	15.88					16.88

D.M.S.: minimum difference significant; C.V. coefficient of variation; Means followed by distincts uppercase and lowercase letters in the row in the column do not differ by Tukey test at the 0.05 significance level.

Table 3
Essential oil yield (kg ha^{-1}) of fresh and dry mass in autumn season

	E.O. Yield fresh mass			E.O. Yield dry mass		
	Grenhouse	**Field**	**Means**	**Grenhouse**	**Field**	**Means**
Mineral	1.672	6.289	3.777a	3.307	9.303	6.628a
Organic	2.209	5.345	3.980a	3.501	9.755	6.305a
Means	1.941 B	5.817 A		3.404 B	9.529 A	
DMS	1.641					3.124
CV%	30.72					35.07

D.M.S.: minimum difference significant; C.V. coefficient of variation; Means followed by distincts uppercase and lowercase letters in the row in the column do not differ by Tukey test at the 0.05 significance level

Spring season

The field presented better production of fresh biomass when compared the greenhouse, in two fertilizations (Table 4). These increments of production had been approximately 151,5% superior in relation to the greenhouse production. For dry biomass no difference was found in statistics between the treatments. The organic and mineral fertilizations can have generated resulted statistical equal in virtue of the good fertility of the soil implanted, that it supplied adequately, the necessities of the plants allowing similar productions. Results for this type of research comparing organic with mineral are changeable with the species.

Table 4
Fresh and dry mass (g parcel^{-1}) of aereal part of *Origanum vulgare* L.in spring season.

	Fresh mass			Dry mass		
	Grenhouse	**Field**	**Means**	**Grenhouse**	**Field**	**Means**
Mineral	615.26	1102.40	858.83a	122.76	127.68	125.22a
Organic	854.28	1124.88	989.58a	132.08	115.40	123.74a
Means	734.77 B	1113.64 A		127.42 A	121.54 A	
DMS	210.14					16.613
CV%	16.51					9.69

D.M.S.: minimum difference significant; C.V. coefficient of variation; Means followed by distincts uppercase and lowercaselettersin the row in the column do not differ by Tukey test at the 0.05 significance level

It is also important mentioning that the most nutrient required by aromatic crops is nitrogen (N), the main representative of the metabolic pathways for the production of essential oil. When this element is already applied by mineralized granular fertilizers such as urea, ammonium sulfate, or associated with phosphate, the capacity of N volatilization or the loss is higher than when supplied through organic compounds. In the latter case, the mineralization of organic matter is slowly and gradually, providing nitrogen around the root system as the need of the plants. Therefore, the production of plant biomass can better respond to the application of organic fertilizers for longer cycles of crops. About the oil contents (%) the results in the field with organic fertilizer were better than greenhouse (Table 5), even without statistical difference.

Table 5
Essential oil content of fresh and dry mass (%) of *Origanum vulgare* L. in the spring season

	E.O. Content fresh mass			E.O. Content dry mass		
	Grenhouse	**Field**	**Means**	**Grenhouse**	**Field**	**Means**
Mineral	0.2978	0.2597	0.2787a	0.854	0.926	0.890a
Organic	0.2540	0.3109	0.2824a	0.849	0.978	0.913a
Means	0.2759 A	0.2853 A		0.851 A	0.952A	
DMS	0.0660				0.436	
CV%	17.07				35.16	

D.M.S.: minimum difference significant; C.V. coefficient of variation; Means followed by distincts uppercase and lowercase lettersin the row in the column do not differ by Tukey test at the 0.05 significance level

The essential oil yield (Table 6) to fresh mass had significant interaction between the factors, observing better yield in the field with two fertilization. The lesser yield was in the greenhouse using mineral fertilization. When the yield means are evaluated in dry mass no difference statistics si founded. As the yield results depend on the values of essential oil content, the statistical differences founded in the yield had been influenced by the production of biomass for area. As in the field the biomass production it was bigger, expected that the essential oil yield is proportional to the increase of biomass for area. In this in case, for the oil yield, the two fertilizations used would be recommended for both system of planting.

These results agree with Carvalho *et al.* (2005), that also did not have influences significant of the organic fertilization and conventional fertilization on the oil yield of *Cymbopogon citratus*. Different results had been found by Resende (2010), where optimum place of production of *Ocimum basilicum* L. with sights to the essential oil yield of fresh mass was in greenhouse.

Table 6
Essential oil yield (kg ha^{-1}) of fresh and dry mass in spring season.

	E.O. Yield fresh mass			E.O. Yield dry mass		
	Grenhouse	**Field**	**Means**	**Field**	**Grenhouse**	**Means**
Mineral	3.83Bb	9.97Aa	6.90a	6.08	6.78	6.43 a
Organic	5.97Ab	8.72Aa	7.35a	5.44	6.81	6.13 a
Means	4.90B	9.35A		5.76A	6.80A	
DMS	1.72			1.12		
CV%	17.54			18.37		

D.M.S.: minimum difference significant; C.V. coefficient of variation; Means followed by distincts uppercase and lowercase letters in the row in the column do not differ by Tukey test at the 0.05 significance level

Essential oil composition

The gas cromatography and the mass spectometer (GC/MS) of essential oil results in 25 chemical constituents. The oil composition was similar for plants cultivated in two systems. It had been identified for fresh and dry biomass, the majority constituents carvacrol, orto-cimeno and terpinen (Figures 1, Tables 7 and 8).

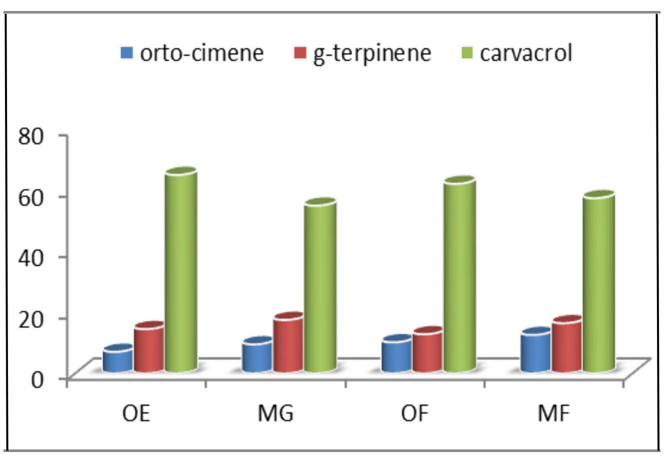
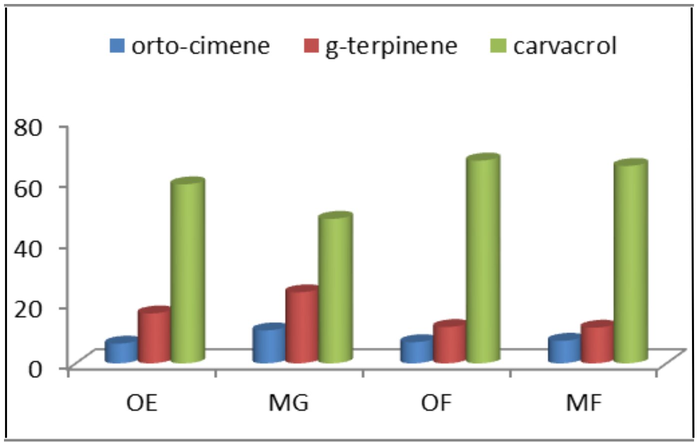
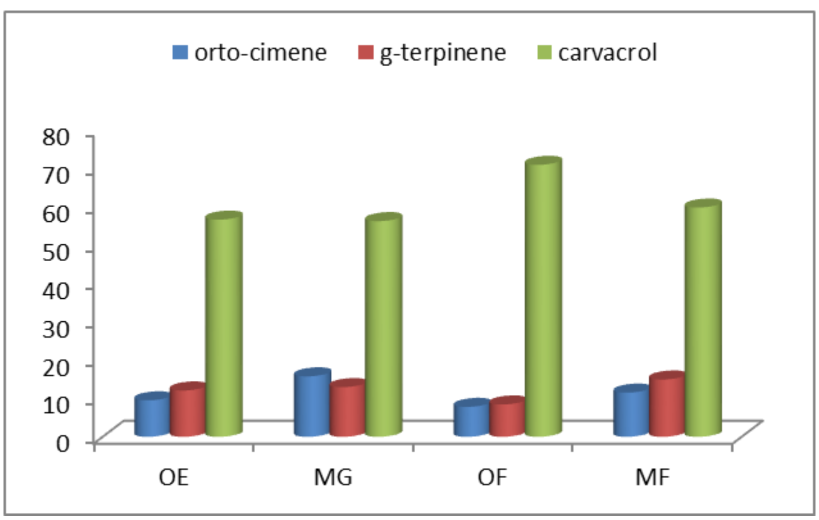


Figure 1

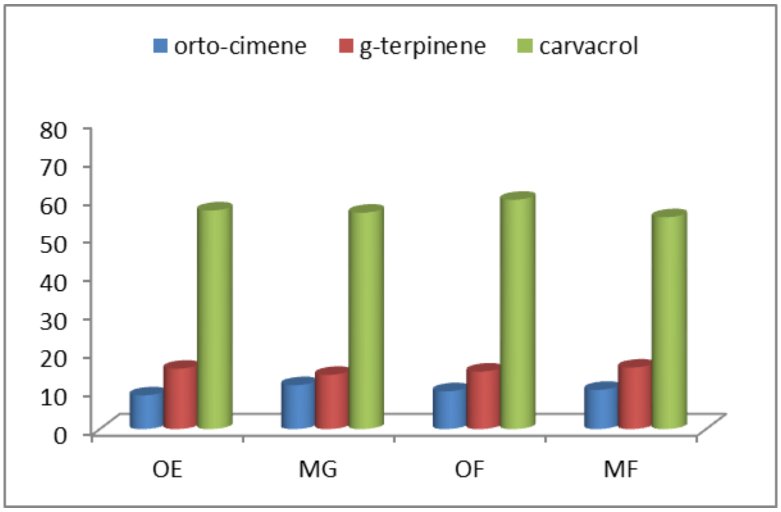


Figures 2

Figures 1 and 2, comparison between the chemical constituent of the essential oil fresh leaf (right) and droughts (left) of *Origanum vulgare* of the season autumn; O.E.: Organic greenhouse; M.G.: Mineral greenhouse; O.F.: Organic field; M.F.: Mineral Field



Figures 3



Figures 4

Fifure 3 and 4. Comparison between the chemical constituent of the essential oil fresh leaf (right) and droughts (left) of Origanum vulgare of the season spring; O.E.: Organic greenhouse; M.G.: Mineral greenhouse; O.F.: Organic field; M.F.: Mineral Field

Table 7
Chemical composition (% relative) of substances gifts in the essential oil of *Origanum vulgare* under interaction of culture systems and types of fertilization at the time autumn

Chemical component	Autumn								IK*	IK**
	Dry mass				Fresh Mass					
	O.G.	M.G.	O.F.	M.F.	O.G.	M.G.	O.F.	M.F.		
α-tujene	0,82	1,64	1,86	tr	1,53	2,23	1,64	2,04	923	931
α-pynene	0,48	0,88	1,12	0,49	0,73	1,00	0,70	0,89	930	939
Canfene	0,19	0,37	0,38	0,27	0,34	0,37	0,21	0,28	945	953
sabynense	nc	nc	nc	nc	tr	0,37	0,20	0,24	969	976
1-octen-3-ol	2,58	1,91	1,73	2,43	1,89	1,83	1,85	3,09	972	978
3-octanone	0,43	0,21	0,33	0,36	0,28	0,25	0,40	0,65	980	986
Myrcene	**2,05**	**2,85**	**2,72**	**2,07**	**2,66**	**2,85**	**2,51**	**2,75**	**987**	**991**
α–felandrene	0,24	0,34	0,29	0,10	0,26	0,34	0,26	0,26	1002	1005
α-terpinene	2,09	2,79	2,28	1,47	2,22	3,22	1,80	1,88	1013	1018
orto-cymene	**7,05**	**9,50**	**10,18**	**12,57**	**6,51**	**10,89**	**6,98**	**7,43**	**1020**	**1022**
Lymonene	0,57	0,87	0,86	0,73	0,58	0,83	0,64	0,63	1025	1031
1,8-cyneol	tr	tr	tr	tr	tr	tr	tr	tr	1028	1033
Z-β-ocymene	0,51	0,85	0,25	tr	3,92	tr	tr	0,29	1033	1040
E-β-ocymene	0,11	0,18	0,10	tr	0,53	0,34	tr	0,13	1043	1050
γ-terpinene	**14,49**	**17,49**	**12,81**	**16,43**	**16,47**	**23,43**	**11,97**	**11,75**	**1054**	**1062**

Nd	0,53	0,33	0,59	tr	0,29	0,56	1,17	0,70	1062	-
Terpinolene	0,11	0,14	0,12	0,28	tr	tr	tr	tr	1085	1088
Lynalol	0,39	0,40	0,38	0,11	0,26	0,29	0,37	0,33	1095	1096
Borneol	0,55	0,79	0,49	0,72	0,54	0,48	0,41	0,45	1161	1165
4-terpineol	0,51	0,58	0,44	0,63	0,32	0,36	0,35	0,32	1173	1177
carvacryl methyl ether	0,31	0,70	0,26	0,40	0,37	1,21	0,63	0,36	1239	1244
timol	0,12	1,74	0,11	2,75	tr	tr	0,12	tr	1287	1290
carvacrol	**64,94**	**54,90**	**62,04**	**57,34**	**59,05**	**47,67**	**66,88**	**65,12**	**1298**	**1298**
E-cariofilene	0,63	0,40	0,34	0,35	0,70	1,02	0,52	0,29	1416	1418
β-bysaboleno	0,16	0,15	0,18	0,25	0,26	0,45	0,30	0,13	1504	1509

O.E.: Organic greenhouse; M.G.: Mineral greenhouse; O.F.: Organic field; M.F.: Mineral Field; (IK*) Index of experimental retention, (IK **) Index of retention literature (Adams, 2007), (tr) Traces of the substance (tr ≤ 0,09), nd) substance not identified, (nc) absence of the substance;

Considering the analyses of oil composition, both systems had favored the production of carvacrol (average generality of 59%), however the treatment with bigger percentage of the constituent was in the field with organic fertilization. This result was waited, since in the previous analyses, because the organic field predominated with better performance in the majority of the agronomics characteristics and in the essential oil.

In relation to the other constituent, terpinene and the orto-cimene had been also favored in both too, with means of 15% for first and 11% for secund. However, no treatment in prominence for these constituent was not observed.

Mockute *et al.* (2001) found different results for plants of *Origanum vulgare* in the District of Vilnius in Lithuania. The chemical composition had disclosed to 42 constituent being the majority beta-ocimene (14 – 21%), germacrene (10 - 16%), beta-caryofilene (10 - 15%) and sabinene (6,6 - 14.2%). In another study with the same species collected during the autumn, in six localities of three distincts geographic areas of Greece, they had disclosed similar majority composites to the present work, being: terpinene (0,6 - 3.6%), p-cimene (17,3 - 51.3%), tymol (0,2 - 42.8%) and carvacrol (1.7% - 69.6%). Plants collected in the Southeast of the country had average between 57,4 the 69,6% of carvacrol. The comparison of essential oils of the collected plants of the same localities that had at the height of summer shown clear differences in the concentration of the four main components, with considerably results than the autumn. In this in case, the Greek oregano is considered generally as the best spice of the oregano, can be unprovided of its commercially valuable when the plants were collected in the autumn (Kokkini *et al.,* 1997).

Seasons analysis

The averages of the temperature during the period of the experiment were minim 20.7º C and maximum 27.3º C and the monthly average precipitation of 114 mm during the months that the culture was in the field. The climate is classified as Aw (megatermic), with two well defined seasons: humid that it is extended of November to March and dry that goes of May to August.

Comparing seasons with systems and fertilization, the leaves fresh biomass have no significant interaction, but in spring season, the field and the organic matter had provided the best conditions for the good development of *Origanum vulgare* L. (Table 9).

Table 8
Chemical composition (% relative) of substances gifts in the essential oil of *Origanum vulgare* under interaction of culture systems and types of fertilization at the time spring

Chemical component	Spring								IK*	IK**
	Dry mass				Fresh Mass					
	O.G.	M.G.	O.G.	M.G.	O.G.	M.G.	O.G.	M.G.		
α-tujene	1,41	0,95	1,26	1,33	2,69	2,22	2,33	2,40	923	931
α-pynene	1,29	0,96	1,11	1,12	1,30	1,12	1,15	1,25	930	939
Canfene	0,51	0,48	0,28	0,33	0,36	0,41	0,38	0,61	945	953
sabynense	nc	nc	nc	nc	0,32	0,28	0,27	0,84	969	976
1-octen-3-ol	2,21	1,98	0,89	2,03	1,92	2,26	1,12	1,41	972	978
3-octanone	0,29	0,34	0,17	0,31	0,30	0,27	0,47	0,31	980	986
Myrcene	**3,02**	**2,15**	**2,11**	**2,30**	**3,39**	**2,61**	**2,66**	**2,79**	**987**	**991**
α–felandrene	0,27	0,23	0,23	0,26	0,32	0,29	0,28	0,29	1002	1005
α-terpinene	2,05	1,93	1,53	2,12	2,50	2,07	2,08	2,16	1013	1018
orto-cymene	**9,49**	**15,74**	**7,79**	**11,51**	**8,81**	**11,47**	**9,88**	**10,24**	**1020**	**1022**
Lymonene	0,70	0,79	0,60	0,72	0,77	0,76	0,83	0,76	1025	1031
1,8-cyneol	0,17	0,24	tr	0,12	tr	0,14	tr	tr	1028	1033
Z-β-ocymene	0,93	1,17	0,86	0,17	1,27	0,93	0,26	0,84	1033	1040
E-β-ocymene	tr	tr	0,15	0,10	0,22	0,24	0,22	0,39	1043	1050
γ-terpinene	**12,06**	**12,95**	**8,42**	**14,91**	**15,75**	**14,14**	**15,01**	**16,05**	**1054**	**1062**
Nd	0,32	0,43	0,52	0,19	0,49	0,26	0,26	0,25	1062	-
Terpinolene	tr	tr	0,12	0,13	tr	tr	tr	tr	1085	1088
Lynalol	0,31	0,42	0,39	0,28	0,27	0,22	0,24	0,22	1095	1096
Borneol	0,70	0,91	0,35	0,43	0,45	0,54	0,42	0,77	1161	1165
4-terpineol	0,47	0,59	0,52	0,49	0,35	0,44	0,33	0,49	1173	1177
carvacryl methyl ether	0,54	0,64	0,40	0,38	0,37	0,42	0,29	0,28	1239	1244
timol	5,55	0,19	0,16	0,14	0,33	1,13	0,20	0,48	1287	1290
carvacrol	**56,58**	**56,19**	**70,86**	**59,73**	**57,03**	**56,44**	**59,82**	**55,25**	**1298**	**1298**
E-cariofilene	0,71	0,43	0,52	0,42	0,58	0,64	0,87	0,70	1416	1418
β-bysaboleno	0,42	0,28	0,23	0,27	0,21	0,36	0,40	0,33	1504	1509

O.E.: Organic greenhouse; M.G.: Mineral greenhouse; O.F.: Organic field; M.F.: Mineral Field; (IK*) Index of experimental retention, (IK **) Index of retention literature (Adams, 2007), (tr) Traces of the substance (tr ≤ 0,09), nd) substance not identified, (nc) absence of the substance;

Table 9
Result of the analyses of plants of *Origanum vulgare* on interaction of culture systems or fertilization with seasons.

	Fresh Mass (g)					
Seasons	**Greenhouse**	**Field**	**Means**	**Mineral**	**Organic**	**Means**
Spring	734.77	1113.64	924.20a	858.83	989.58	924.20a
Autumn	241.77	585.38	413.58b	414.26	412.89	413.58b
Means	488.27B	849.51A		636.54A	701.23A	
DMS season =	132.02			132.02		
	DMS system			**DMS fertilizantion**		
CV%	21.53			21.53		

D.M.S.: minimum difference significant; C.V. coefficient of variation; Means followed by distincts uppercase and lowercase letters in the row in the column do not differ by Tukey test at the 0.05 significance level.

Table 10
Result of the analyses of plants of *Origanum vulgare* on interaction of culture systems or fertilization with times

	E.O.Yield dry mass (kg ha^{-1})					
Season	**Systems**			**Fertilizantion**		
	Greenhouse	**Field**	**Means**	**Organic**	**Mineral**	**Means**
Spring	6.80 Aa	5.76 Ab	6.249	6.13	6.43	6.249a
Autumn	3.40 Bb	9.52 Aa	6.467	6.62	6.30	6.467a
Means	4.747	7.969		6.46A	6.25A	
DMS season =	2.0945			2.0945		
	DMS system			**DMS fertilizantion**		
CV%	35.94					

D.M.S.: minimum difference significant; C.V. coefficient of variation; Means followed by distincts uppercase and lowercase letters in the row in the column do not differ by Tukey test at the 0.05 significance level

The oil yield of fresh biomass did not have significant interaction between seasons, system of planting and fertilizers (Table 11). When observes the independent effect, the field presented better resulted than greenhouse during the spring. However, the greenhouse had the worse performance at the autumn.

Particularly, the development of glandular trichomes that produces structures and store the essential oil is required more light (Morais, 2006). So, the increased production of secondary metabolites under high levels of solar radiation, like the spring season, is explained by the fact that the biosynthetic reactions are dependent upon supplies of carbon skeletons performed by photosynthetic process and energy compounds that participate in the regulation of these reactions (Taiz & Zeiger, 2006).

On the order hand, most internal temperature of a protected agricultural environment (greenhouse) is due to the greater spread of radiation and retention of solar radiation into the environment, with lower incidence of direct radiation, an ideal advantage for places with very cold weather or very low temperatures. In other aspects, this can reduce the cycle of the plants and provide faster and early harvest and production results. In the greenhouse, there is also an influence on the humidity, but because of less ventilation in the indoor environment, this provides greater moisture from evapotranspiration of plants and irrigation in relation to the external environment (Andriolo *et al.*, 2005).

Table 11
Result of the analyses of plants of *Origanum vulgare* on interaction of culture systems or fertilization with seasons

	E.O.Yield fresh mass (kg ha^{-1})					
	Systems			**Fertilizantion**		
Season	**Greenhouse**	**Field**	**Means**	**Organic**	**Mineral**	**Means**
Spring	6.263	9.961	8.112 a	8.806	7.418	6.291a
Autumn	1.941	5.817	3.879 b	3.777	3.980	5.699a
Means	4.102B	7.889A		6.291A	5.699A	
DMS season =	1.496			1.496		
	DMS system			**DMS fertilizantion**		
CV%	27.23					

D.M.S.: minimum difference significant; C.V. coefficient of variation; Means followed by distincts uppercase and lowercase letters in the row in the column do not differ by Tukey test at the 0.05 significance level

Like another studies, it can be inferred that aromatic plants the production of secondary metabolites depends on the genetic control and also of genotype and environment interactions, which can be triggered by stress conditions, ie, excess or deficiency of some environmental factor, such as light, temperature, nutrients, among others. In the case of oregano, the higher light intensity during the spring and an adequate supply of nutrients throughout the experiment period were the factors that ensured greater biomass production and consequently, higher oil yield.

It can be concluded that the field had greater favored oil yield and rates of carvacrol, and the biomass production was better at the spring mainly using organic fertilizer.

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