Oreganum vulgare; techno – economic analysis for the alternative uses and the role of soil – climate conditions

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Abstract. The connection between research activity of higher educational institutions and productive institutions plays a special role in the economic development of local communities. The exploitation of research results in areas such as the primary sector enables the development of innovative projects in the rural economy, as well as in the energy sector. In the current study, the research activity in the field of medicinal plants as a dynamic form of cultivation is presented. Our research refers to the qualitative and quantitative composition of distillate aromatic plants (flowers).The results can be reflected in the primary sector of Western Macedonia, in order to apply appropriate soil and climatic conditions in the region and to develop new crops adjusted to the specific conditions, especially in the field of medicinal plants. The proposal in cooperation with the Local Cooperative of Aromatic and Medicinal Plants has been presented in a series of workshops and has been utilized appropriately to strengthen rural culture in the region. The need to deal with the particular crop stems from the fact that the final products (oil and water) are products that may have direct application in the food sector, cosmetics, aromatherapy, human and animal health.

1. **Introduction**

Origanumvulgare ssp. hirtum is internationally known as Greek oregano (English Greek oregano), which is native to our country. It is a plant with a very flexible developmental character in terms of

soil requirements. It appears in a wide variety of soils and climates, from coastal to mountainous areas, from islands to mainland Greece, in rich and poor soils. Oregano with stands drought and can be grown arid. However, in prolonged drought, especially during the period of spring, watering it once or twice (30-40 mm each time, corresponding to 30-40 n.M./Ha.) can be beneficial, increasing the efficiency without particularly reducing the quality.

Oregano is multiplied by seed or by division of suckers and plants taken from old plantations. Plantation may be performed during the fall as well as during spring. In order to obtain seedlings for autumn plantation installation, seedbeds get prepared during late July to early August, while for spring crops the preparation is done during January, according to the local climatic conditions, and even better in specially designed lawns under cover in fare.

The planting of seedlings or cuttings can be fulfilled during two periods: in October or in March. If it is possible, the planting drip irrigation can be performed until mid-May. The field should be properly prepared for the growing. Seedlings or shoots are planted at a distance of 60 to 80m between the rows and 30 to 40m between plants in line. In arid and weak (infertile) fields, it is planted densely while in loudly (fertile) fields or when the crop can be helped with some watering, less so. The plantation should be kept clean of weeds until summer. Therefore, treads are required between the lines, carved with a special machine (as in tobacco culture) and between plants, made with hoe by hand.

Harvesting is done once at full flowering by cutting all the exposed parts at a height of 8-1cm from the ground. The plantation reaches full production in its third year and continues with a stable rate for another 3-4 years.

The cutting is done with a trimmer. Oregano of the best quality can be acquired when the cut mass is transported loose loaded in special drying machines or when it is dried in the shade.

The mass can also with adjust to the combine harvester threshing on the spot in the field, by being already drained after cutting and by being kept in sacks. Then depending on the direction of the product to the market, it is either sieved and left with the leaves and flowers, destined for medical consumption (drug) or distilled threshed as it is intended for the production of essential oil of oregano. The utility of this product is either commercial, i.e. pure for direct consumption in the population or for medical use in the manufacture of medicaments.

In poorly drained farmland during the cultivation of oregano, rot of roots may be present, caused by soil fungi; for this reason, cultivation of oregano in such farmland should be avoided.

Figure 1. Oreganumvulgare, a vibrant rural culture, which finds application in the region of Kozani, Greece.

In a dry form (leaves, flowers),the oregano is used in cooking, as well as in the essential oil and water, but in small portions. Especially the essential oil finds applications in feed for pigs, poultry and lamb serving as a natural antibiotic. Also strong antioxidant, anticancer, antibacterial and antiseptic properties of oregano (dry, medical drug, essential oil, oregano water) make these products precious good health advice and taste improvement in human nutrition [1-4]. In our research, a complete production management system and a dynamic rural culture of the oregano is presented, which finds application in the region of Kozani, Greece (Figure 1).

An attempt to present the cultivation, harvesting, production process and analysis of plant distillates properties is made, through specific soil and weather parameters that make it environmentally friendly.

This type of crop is presented in this survey taking into account all the features such as soil analysis, distillation and the process through which oregano essential oil is produced. With regard to the essential oil of oregano, it was necessary to present its chemical composition and the flavorings contained therein. But in addition the oregano essential oil, the process of oregano water production is presented, followed by the analysis of physicochemical and microbial parameters. The above research is presented by Constantinos Tsanaktsidis, Eleni Zafeiriou, Konstantinos Spinthiropoulos, George Kasapidis, and Aikaterini Itziou.

Based on the above, conclusions are drawn about the type of the proposed cultivation, as environmentally friendly, and the quality characteristics of the products (oregano water and oregano oil) that make them competitive for potential use and applications in everyday human life.

2. Materials and methods

In order to move on to our empirical research, a Soil analysis is presented. The Soil Analysis was performed in a representative sample of one field. We chose a sample of soil from the whole area, since the soil is similar in the whole area. The Soil analysis was determined by the methods [5-6], Qualitative method modified [7-8], Practical Handbook [9].

Figure 2. Presentation of the distillation process for the production of oregano essential oil.

The dried oregano that came from the flower of oregano is put in the retort in a dry form (the whole shaft and the ground). Then, it is cut with a specific machine and left for two days in the field to dry, in order to then be gathered in large sacks (we can keep them in storage for several days). Then the oregano is put in the cauldron (a cauldron of 4000 liters holds around 500-600 kg of dry oregano and about 700 -750 kg of milled oregano). The distillation process lasts for about 3 hours. The distilled product (water and oil) is fed into a specific container where two tiers and based on the difference in specific gravity between the oil and the water, the oil is separated from the water. Therefore, the top tier is occupied by the water and the lower one by the oil. The capacity of the tank is 100 liters and usually about 70 liters of the distilled product are pumped in as we can observe on Figure 2.

In our effort to determine both the chemical composition and the composition of the existing aromatic substances we used a specific sample. This sample of diluted essential oil (1/10 in n-heptane, v/v) for the GC-MS type assays. It is worth mentioning that these analyses were done by using the Agilent 5975C Series GC-MSD (7890A GC and 5975C dormant MSD) system. This system operated in an EI state at 70 eV, equipped with a capillary column HP-5M5 (30 m x 0.25 mm, film thickness 0.50 μm). In the process, 2 ml of dilute essential oil was introduced (separately in a split and splitting ratio 50:1). Throughout the experiment, the inlet temperature remained constant at 250 ℃ and Helium was used as a carrier gas at a constant flow rate 1.2 ml / min It should be noted that the oven temperature was as follows: 40 ℃ for 10 minutes, raised to 220 ℃ (4 ℃ / min) for 10 minutes and raised to 320 ℃ (5 ℃ / min) for 20 minutes The MSD operates in a scanning operation in the 50-700 m / z range.

Continuing our analysis, mass spectra and the known RI index were compared to NIST (National Institute of Standards and Technology, Gaithersburg, MD, USA, NIST 05 and NIST 05 s). Based on the peak areas of the known GC integrator, the individual components were calculated. At this point, we have to mention that the whole process proceeded without the use of corrective factors.

The comparison process is generally known. The relative retention index (PH) and mass spectra were decided to compare with those of the NIST commercial spectral library. In order to be able to calculate the relative amounts, we have taken into account the peak areas of the well-known GI integrator. The whole procedure was done without the use of corrective factors

The process gave a complex range of organic compounds mainly terpenes. This analysis did not detect any components present at very low concentrations.

The microbiological and physicochemical analyses in a sample of oregano water were determined with the methods: "ISO 11290-2, ISO 16649-2:2001, ISO 21528-2:2004 ,ISO 6888-2, ISO 6222:1999".

3. Results and discussion

3.1. Soil analysis

In Table 1 we can see that the soil analysis was done in a sample (representative) of a field.

Table 1. Soil analysis by using a representative sample.

a/a	Parameters of our analysis		Methodology used
1	pH	7,26	ISO 10390:2005[5]
2	Conductivity (μS/cm)	145,3	ISO 11265:1994
3	Mechanic constitution	Characterization: ammopilodes of sand	Qualitative method modified from The, 1979
	of soil	50-70%. Sandy loam (SL)	
4	Nitrogen (%w/w)	0.074	ISO 11261:1995[7]

5	Phosphate(mg/kg)	22.03	ISO 11263:1994[8]
6	Potassium (mg/kg)	88.67	Practical Handbook N.T. Faithfull 2002, CABI Publishing
7	Cuprate(mg/kg)	1.02	ISO 14870:2001[9]
8	Zinc (mg/kg)	3.18	ISO 14870:2001[9]
9	Maganese (mg/kg)	7.09	ISO 14870:2001[9]
10	Ferrate (mg/kg)	13.61	ISO 14870:2001[9]
11	Boron (mg/kg)	0.18	METHODS OF ANALYSIS FOR SOILS, FAO, 2007

3.2. Oregano water composition analysis via GC/MS

We can observe the results of the physicochemical properties analysis of oregano water in Table 2. In each case we noticed that there is no concern about these parameters.

Table 2. Analyses and Results of physicochemical attributes in a sample of oregano water.

Analysis Of Physicochemical Properties	Result	Units	Ref. Values
Pη	4.42		
Conductivity	62.8	μS/cm	<2000
Total Hardness	0.5	d0	
Nitrates	3.8	Mg/lt	50
Nitrate	0.03	Mg/lt	0.5
Ammonia	0.11	Mg/lt	0.4

The results of the microbial content for the first sample of 25ml are showed in the Table 3 below. Taking into account these results, we could mention that there is no problem, as far as these parameters are concerned. At this point, it should be stressed that in order to avoid possible errors in our measurements, we repeated the experiment in a subsequent period and the results were the same. Taking into account the results, it can be ascertained that there is no possibility for microbiological change. Chances remain extremely low even during storage of the oregano oil (small bottles of 25ml).

Table 3. Microbiological results and analyses in a sample of oregano water.

Sample	16/4-6-2015/364	Units	Ref. values	Method
Salmonella spp	-	-	-	ISO 6579:2002
Listeria monocytogenes	-	-	-	ISO 11290-2
Count for positive to β-glycuronidase Escherichia coli	0	Cfu/ml	0	ISO 16649-2:2001
Enterobacter count	0	Cfu/ml	0	ISO 21528-2:2004

Staphylococcus aureus	0	Cfu/ml	0	ISO 6888-2
O.M.X. 30 0 C	0	Cfu/ml	100	ISO 6222:1999

3.3. Essential oil composition analysis via GC/MS

The results obtained from the analysis of oregano oil gave spectrums of all the detectable compounds listed below. Using the Agilent 7890A Gas Chromatograph / 5975C Mass Selective Detector System we received the following results (Figures 3-5) and (Table 4).

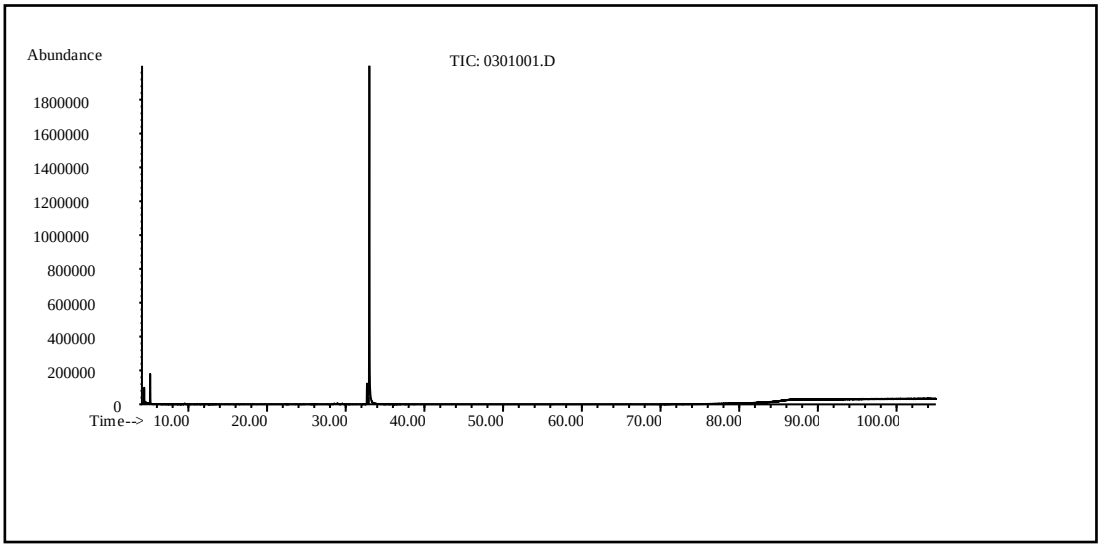

Figure 3. TIC (total ion chromatogram): Aqueous solution containing carvacrol. TIC organic extract (dilution 1/500).

The use of the GC-MC method lead to the detection of carvacrol, which is the major compound (67.34%) and thymol (6.94%), p-cymene (5.72%) and γ-terpinene (5.21%) , β-caryophyllene (RT: 37.12) 1.97% and β-bisabolene (RT.: 39.76) 1.95%. .They are listed below (Figure 4).

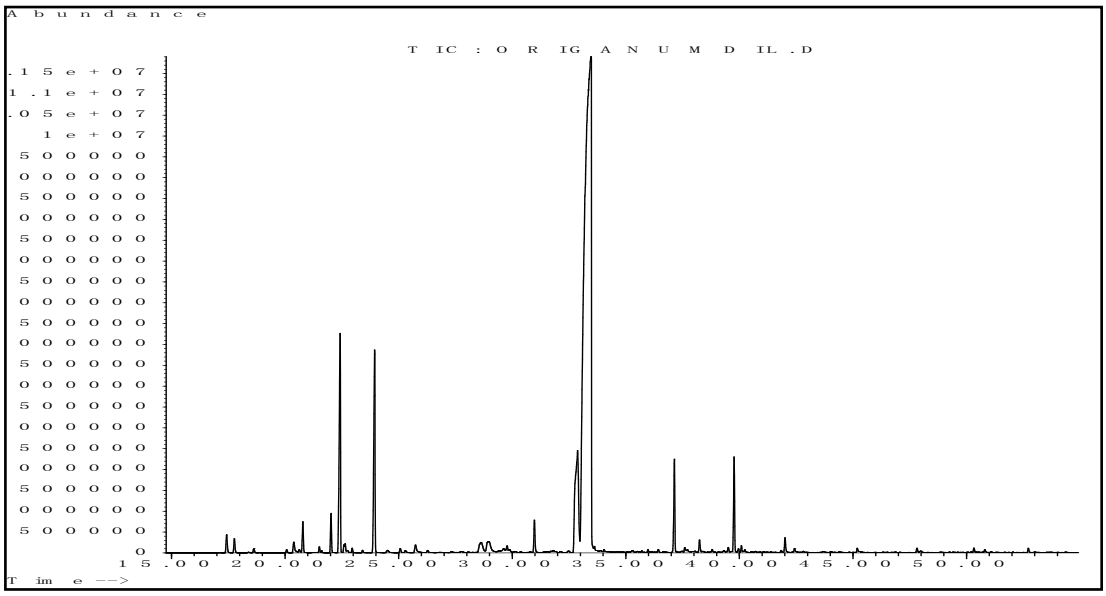

Figure 4. The GC-MS chromatogram for the sample Spec oregano essential oil.

It is known that when we have Lower essential oil concentrations then we can gain better peak shapes. It should also be mentioned that in cases such as the one described above it is more efficient to separate the components, but compounds present in a small percentage may not be detected. (Figure 5)

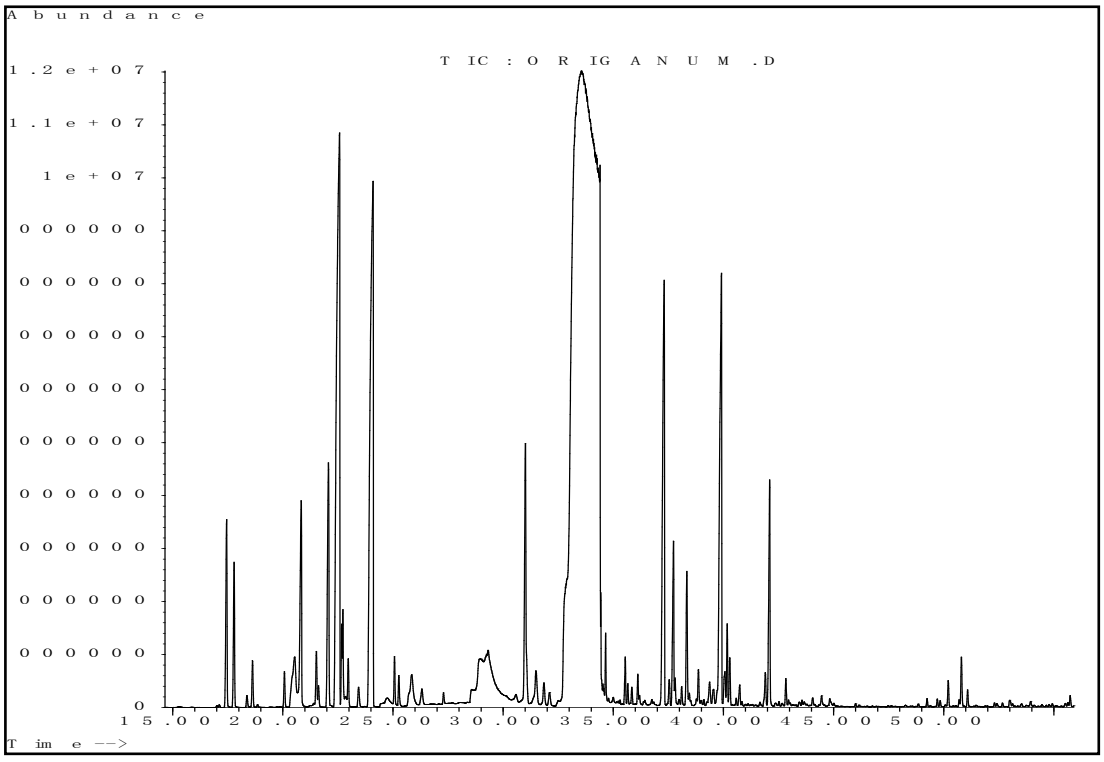

Figure 5. Spectrum analysis with oil sample gas chromatograph from oregano distillation**.**

Table 4. The major compounds of oregano oil as analysed with Gas Chromatograph/5975C Mass Selective Detector.

A/A	RT	Compounds	%	A/A	RT	Compounds	%
1	17.44	α-Thujene	0.85	20	29.33	cis-β-Terpineol + Borneol	3.64
2	17.79	α-Pinene	0.62	21	31.02	Thymol methyl ether	1.54
3	18.62	Camphene	0.21	22	31.50	Dihydrocarvone	0.39
4	20.07	β-Pinene	0.16	23	32.87	Thymol*	*
5	20.52	1-Octen-3-ol	0.52	24	33.44	Carvacrol + Thymol	60.10
6	20.82	Myrcene	1.20	25	35.53	Thymolacetate	0.40
7	21.52	à-Phellandrene	0.38	26	36.12	β-Bourbonene	0.27
8	21.62	3-carene	tr	27	37.29	β-caryophyllene	2.98
9	22.06	à-terpinen	1.30	28	37.52	Germacrene	tr
10	22.51	p-cymene	7.27	29	37.73	unknown	1.02
11	22.66	Limonene	tr	30	38.34	α-Humelene	0.82
12	22.74	β-Phellandrene	tr	31	38.87	ç-Muurolene	0.28
13	22.97	trans-β-ocimene	0.20	32	39.36	Varidiflorene	tr
14	23.44	cis-β-ocimene	0.13	33	39.87	β-Bisabolene	3.87
15	24.04	γ-Terpinene	5.84	34	40.04	τ-Cadinene	tr
16	25.07	Terpinolen	0.36	35	40.17	δ-Cadinene, (+)-	0.53
17	25.27	àp-Dimethylstyrene	Tr	36	40.29	β-Sesquiphellandrene	tr
18	25.84	β-Linalool	0.49	37	41.90	Spathulenol	tr
19	28.90	Borneol *	*	38	42.08	Caryophyllene oxide	1.20

Environmental conditions, such as soil quality, sun exposure, temperature etc, may also contribute to the determination of phenolic concentrations [10-13]. The empirical analysis of this work has taken into account the international literature in order to ensure its validity. Older empirical studies [4, 10-11] have shown that the presence of a carbamole / thymol chemist of Origanum vulgare ssp. hirtum was positive. The qualitative composition of oregano has been the subject of several studies, however, it has not been connected either with the composition of the soil where it is grown or with the production of a secondary product, the oregano water. In the present work, these parameters and indeed presented, and, what is more, a high percentage of carvacrol as a component of oregano oil is found, which has not been reported in the previous literature in such high concentrations.

3.4. Economic analysis of oregano cultivation

The oregano belongs to the most profitable variety of aromatic plants while its use is not limited to the traditional one but also serves as medicinal syrup. The two alternative uses are profitable with different production costs while in case they are certified as organics, they provide the greatest revenue. A major part of the total production is exported to major destination countries such as the USA and Germany.

In order for an investment in Oreganum vulgare to be promoted, the classic method of net present value may well be employed while the destination of its cultivation should also be taken into consideration. Furthermore, the profitability of the particular cultivation, as mentioned above, does not only depend on annual income but it takes two or three years to reach its maximum profit, in the third year and, therefore, a ten-year time period should be selected in order for a real perception of profitability to be formed.

First of all, the settlement cost reaches 220 euros per acre, with all the expenditures included, while the price per kg ranges from 1.8 to 2.3 euros for a conventional crop. On the other hand, in case of an organic crop, the price can even reach six euros per kg. As an alternative use, the production of oregano oil can be selected with a proportion of 7% of the total production. The role of irrigation conditions is substantial since it may well lead to a productivity increase per acre. Furthermore, the income generated by oregano oil reaches 70 euros per liter or 300 euros per acre. As far as revenues per acre are concerned, they can reach 400 euros per acre (for the classic use) while a farmer's revenue may become 1000 euros in case of an organic certified cultivation. Sustainability for the particular cultivation asks for a minimum price of 0.7 euros in order for the production costs to be satisfied.

For the aforementioned reasons we employ the methodology of net present value for a time period of ten years. In year 0 the settlement of the crop occurs, while the profit increases gradually and its greatest value is reached in the third year. The formula used in the particular methodology is the following;

$$NVP = \sum_{t=1}^{10} \frac{\Pi_t}{(1+r)^t}$$

where t denotes the year whose cash flow is being discounted, Π denotes the cash flow for each year,R denotes *Discount rate*.

As mentioned above and according to the production costs and the prices per kg used, the cash flows are formed as follows for the case of the dry oregano;

$$\Pi_0 = -220,\ \Pi_1 = 200(2{,}5 - 07) = 360,\ \Pi_2 = 250(2{,}5 - 0{,}7) = 450,$$
$$\Pi_3 = 300(2{,}5 - 0{,}7) = 540,\ \Pi_{4-10} = 540$$

For our case r is equal to 5%.

According to our findings NVP=4665,714 (per acre).

The investment for the oregano crop if the production is for oregano oil is good, because the cash flows associated with the net income obtained reaches EUR 300 per acre. The calculation of the NVP in this case gave us the following results.

$$\Pi_0 = -220, \Pi_1 = 300,\ \ \Pi_{2-10} = 300,$$

For the same discount rate the NVP =2637,43. What is evident is that the net income that comes from oregano oil for the producer is almost half despite the difference in price per kilo.

4. Conclusions

The essential oil of the Greek oregano consists of individual associations, while two of the most important are thymol and carvacrol. Thymol (2-isopropyl-5-methylphenol) is an oxygenated monopropene, and a phenol derivative of cymene. The carvacrol ($C_6H_3CH_3$ (OH) (C_3H_7)) is a monoterpenoid phenol.

The thymol, due to antifungal, antioxidant and antibacterial properties, is a component of many commercial preparations such as mouthwashes, toothpastes, soaps, shampoos, deodorants and medications for cough and cold. In fact, laboratory experiments performed on mice showed anti-inflammation and a better healing process. Also, plants containing thymol are used both as aromatic and in cooking for the characteristic smell of thyme.

The carvacrol may inhibit the growth of several strains of bacteria. It is often used as a food additive for the prevention of bacterial contamination because of its low toxicity and its pleasant taste and smell. The antimicrobial properties are due to the breakdown of the bacterial membrane. The

carvacrol is easily metabolized and excreted from the body. It could be supported that the properties of carvacrol, in combination with the low PH levels of the oregano oil (4,42) may contribute to its use as a preservative in food chemistry. Moreover, this high concentration of carvacrol in oregano oil is considered the most important characteristic of the high quality of the specific oregano type.

It has been supported that Oreganum vulgare ssp. hirtum may direct its biosynthesis to the formation of a certain compound that is considered preferable. More specifically, the metabolic pathway of phenolic compounds is through the autooxidative conversion of γ-terpinene to *p-cymene* followed by hydroxylation of *p-cymene* to thymol or carvacrol [10].Compared to the essential oil produced by Rosa damascene, thymol and carvacrol are detected in both oils [14]. However, the two oils are very different, a fact that may well be attributed to the different concentrations of these substances that are found in the two oils.

Finally regarding the economic issues of the two alternative uses in terms of producer profit and for a ten year time period, the dry oregano should be preferred over the oil given the high extraction cost. An issue left unanswered is the case of organic oregano that is much more profitable than the conventional crop.

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