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Short Review

Ecology, Cultivation and Utilization of the Aromatic Greek Oregano (*Origanum vulgare* L.): A Review

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Abstract

Medicinal and aromatic plants represent a stable part of the natural biodiversity legacy of many countries in the world. The present review focuses on oregano (*Origanum vulgare* L.; family Lamiaceae), an endemic herb in Greece that constitutes one of the best known aromatic and medicinal plants originating in the Mediterranean region. In particular, oregano is an evergreen, rich in natural compounds perennial plant that received increased attention in the last years for a wide range of uses. Oregano dry leaves and inflorescences in mixture are used as human and animal food that is extremely rich in antioxidative properties. Additionally, its essential oil is rich in carvacrol, thymol, c-terpinene and p-cymene, and is used for a number of medicinal purposes, e.g. for inhibiting microbial and fungal toxin production as well as for the well-known anti-inflammatory, analgesic, antiarthritic, antiallergic, anticarcinogenic, antidiabetic, cardioprotective, gastroprotective, hepatoprotective, and neuroprotective properties. Due to its perfect quality and high essential oil concentration, Greek oregano (*O. vulgare* ssp. *hirtum*) is regarded among the best in the world gaining in popularity in the global markets for food industry applications. Consequently, oregano might be considered as an important low-input, environmental friendly commodity for extensive cultivation in Greece. The present review summarizes on the origin, the morphology, the ecology and the utilization of this plant. Despite the extensive literature available on the use of oregano biomass and essential oil, only few reports exist concerning the cultivation of this plant. Therefore, the present review is additionally focused on the cultivation practices and the importance of cultivation and utilization of *Origanum vulgare* L. in Greece and generally in the Mediterranean region in the near future, as it constitutes a plant species with high medical, economic and environmental value.

Keywords: cultivation practices; Greek species; multipurpose herb; oregano oil

Introduction

The Mediterranean Basin is considered as a 'Global biodiversity hotspot' and the largest of the world's five Mediterranean type climatic regions (Beltran *et al*., 2014; Solomou *et al*., 2017). The geographical position of Greece combined with its richness in different landscapes and edapho-climatic environments and their multiple interactions with the biotic factors have designated it as a region of great importance regarding biodiversity and plant endemism, particularly favouring the adaptation of aromatic and medicinal plant species that offer therapeutic, economic and environmental benefits (e.g. *Origanum* sp., *Sideritis* sp., *Salvia* sp., *Crocus* sp., *Hypericum perforatum*, etc.) (Katsiotis and Chatzopoulou, 2010; Solomou *et al*., 2016).

Oregano constitutes one of the most cultivated aromatic plants worldwide. Due to its perfect quality and high essential oil concentration, Greek oregano is regarded among the best in the world gaining in popularity in the European markets for food industry applications (Goliaris and Skroumpis, 1992). Depending on the origin, the essential oil concentration ranges from 1.1 to 8.2% that is about 10 times greater compared to other oregano species (Kokkini and Vokou, 1989; Baser *et al*., 1993).

Greek oregano grows particularly on mountains and hills, but the plant started to be cultivated in many Greek lowlands, as for example in the plains of Karditsa, Trikala, Magnisia, Rodopi, Kilkis and Thessaloniki. Greek oregano has also been detected in the Balkan Peninsula, e.g. in Turkey, Cyprus, Italy (Sicily) (Siedemann, 2004), particularly at elevations between 0 and 1500 m ASL (Kokkini *et al*., 2004).

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Goliaris *et al*. (2002) created four new oregano clones in Greece from indigenous plant material (varieties) grown in North and Central Greece (see Fig. 1). The criteria on which the selection was based comprised plant shape, bloom time, yield, essential oil content and dry plant color.

Despite the wide research on the essential oil concentration and components as well as the secondary metabolites of the *Origanum vulgare* L. (Dhifi *et al*., 2016), only few reports exist concerning the cultivation practices of this plant. Therefore, the aim of the present review is to present comprehensive information about the importance of cultivation and utilization of *Origanum vulgare* L. in Greece and generally in the Mediterranean area, as it constitutes a plant species with high medical, environmental and economic value.

Methodology

A literature search was conducted using Scopus, Web of Science and Google Scholar, with the aim to review and consolidate the existing research on the cultivation and utilization of *Origanum vulgare* L. in Greece. A systematic methodology was applied so as to ensure a rigorous and repeatable method of cultivation and utilization of *Origanum vulgare* L. The methodology consisted of the following phases: (a) the generation of keywords and (b) a systematic search (Harrison *et al*., 2014).

Oregano types and plant morphology

Oregano belongs to the Lamiaceae family of the order Lamiales (Makri, 2002). The genus *Origanum* includes more than 70 species, 49 taxa (species and subspecies) and natural hybrids found in almost all Mediterranean countries, but also in the temperate zones of Asia and America (Kintzios, 2002). The most important types of oregano are: a) Greek oregano (*Origanum vulgare* ssp. *hirtum*), b) Spanish oregano (*Coridothymus capitatus* L.), c) Turkish oregano (*Origanum onites* L.), d) Mexican oregano (*Lippia graveolens* HBK (Lawrence, 1984), e) *Origanum vulgare* L. ssp. *vulgare,* f) *Origanum vulgare* ssp. *viridulum,* and *g) Origanum vulgaris* L. (Kokkini and Vokou, 1989).

Generally, oregano plants grow 20-90 cm in height and are characterized by egg-shaped leaves 10-40 mm long and 5-25 mm wide, with the wider end at the base, and with smooth or very shallowly toothed edges and leaf tips varying from acute to round-shaped. The inflorescence is many-flowered, with flowers grouped into short dense lateral or terminal spikes. The corolla (ring of united petals) is white to purplish, 4-8 mm long, and has two lips. The calyx (ring of united sepals) is five-toothed. Each flower has four stamens (male parts). Each fruit has four small nutlets (single-seeded units).

A number of studies have shown that variation in morphological and chemical features may occur within a single oregano species. It has been found that the pattern of variation of a single species follows its geographical distribution or it depends on the season of plant collecting. The range of three subspecies found in Greece is associated with the climatic conditions prevailing in each area. *O. vulgare* subsp. *hirtum* (syn. *O. hirtum* Link*, O. heracleoticum* auct. non L.), is mainly found on the islands and southern mainland (Fig. 1), whereas toward the north it is mostly confined to the lowland coastal areas. Its distribution range in Greece is limited by the presence of the continental type of climate in the northern and central part of the mainland (Kokkini *et al*., 1991). From the morphological point of view, subsp. *hirtum* can be distinguished by its small green bracts and white flowers. Toward the northern parts of Greece, where a continental Mediterranean climate occurs, subsp. *Oregano hirtum* is replaced either by subsp. *viridulum* [syns. *O. heracleoticum* L., *O. viride* (Boiss.) Halácsy] characterized by large green bracts or by subsp. *vulgare*. The latter is easily distinguished by the large purple bracts and pinkish to purple flowers. The number and the size of the sessile glands in leaves, bracts and calyces are remarkably reduced in samples from the southern to the northern part of the country. These glands which appear as small bladders are the peltate glandular hairs described by Bosabalidis and Tsekos (1984), Werker *et al*. (1985). Since they contain the bulk of the secreted essential oil, the reduced number of sessile glands is connected with low essential oil content (Bosabalidis and Kokkini, 1996). The subsp. *hirtum* plants, though very variable in leaf and bract sessile gland number, but always characterized by densely glandular calyces, are in any case rich in essential oil (1.8-8.2 ml/100 g dry weight). On the other hand, plants belonging to the other two subspecies, having fewer and smaller (inconspicuous) sessile glands, contain a much lower amount of essential oil (traces up to 0.8%) (Kokkini and Vokou, 1989; Kokkini *et al*., 1991, 1994).

Crop ecology and cultivation practices

The wild oregano species can grow at different altitudes from coastal to mountainous areas in undulating to rolling terrains (Goliaris, 1997b) on various soils with different fertility status, able to withstand rather low temperatures, and finally produce high yields of good quality. However, *O. vulgare* ssp. *hirtum* may be extensively cultivated and play a very important role in the Greek economy.

Climate and soils

A number of relevant studies including the effects of temperature and day length have been conducted by various authors (Cohen *et al*., 1980; Putievsky, 1983; Dudai, 1988; Putievsky *et al*., 1988; Dudai *et al*., 1989; Dudai *et al*., 1994). According to these studies, air temperature for optimum oregano growth and development range from 18 °C to 22 °C, whereas the root system of well-developed plants (older than one year) may withstand air temperatures from -25 °C to 42 °C (Koutsos, 2006). However, temperatures below 4 °C or above 33 °C may limit plant growth. The plant grows in a wide variety of soils and climates from seaside to mountainous areas on the islands and mainland of Greece (1500 m), in rich as well as on poor calcareous soils. An excellent soil pH value is around 6.8, but oregano may be found on calcareous soils with much higher pH values. Generally, a long light period is needed (more than 12 hours) for a high content of essential oil and carvacrol.

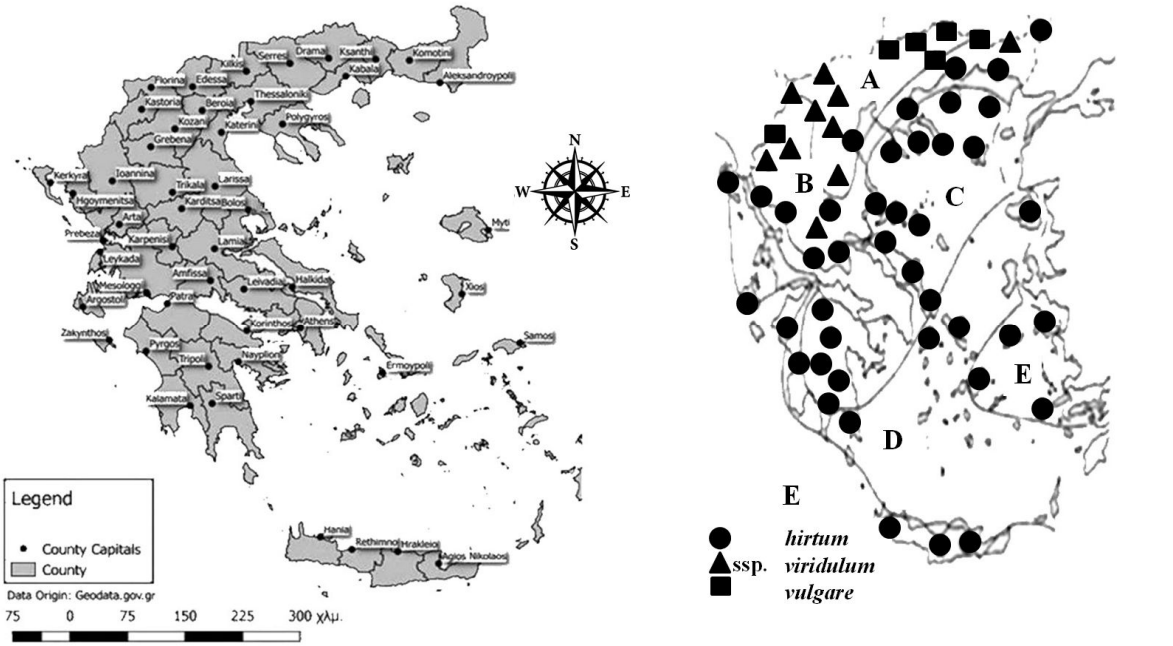

Fig. 1. *Origanum sp*. distribution in Greece (Kokkini *et al*., 1994)

Moreover, as many other species, oregano plants grown under different temperature or light regimes flower earlier as temperature and light increase.

As the day becomes longer – due to natural or artificial light – essential oil content increased and its composition changed. On the basis of these results it is apparent that the origin of this variation is linked to changes in the photosynthesis of the plant. There is a relationship between number of leaves per plant and plant parts and the essential oil content and composition, as well as between the number and type of glandular hairs (Werker *et al*., 1985a, 1985b; Dudai *et al*., 1988c).

Field preparation and planting

A deep winter ploughing is advisable followed by a light tillage just prior to planting. Before planting, a final plowing is suggested to cover the fertilizer and for the destruction of weeds, and to facilitate the transplantation, particularly when this is done mechanically.

Greek oregano is primarily collected from wild populations. The *O*. ssp. *hirtum* has great variability in the essential oil content and carvacrol percentage, so clones of high yield and quality were developed for introduction into cultivation.

As oregano seeds are very small (0.20-0.25 g per 1000 seeds), direct sowing in the field is not recommended for commercial cultivation because of the generally low germination rates, the heterogeneity of the young plants and the considerable delay in yield production (Putievsky *et al*., 1982). Instead, seedlings are prepared using rooted or unrooted stem cuttings (Kuris *et al*., 1981). The seedlings are transplanted in the field in the period March-April. Plant density greatly affects plant yield and number of branches (Marzi, 1996). Field experimental results have shown that planting in rows with about 8-10 plants/m^2 might be optimum, with distances 60-80 cm between the rows and 30-40 cm on the row (Katsiotis and Chatzopoulou, 2010) for proper hoeing and mechanical weeding.

Fertilization - Irrigation

High dry matter yields are associated with increased N-P-K dressings. According to Dordas *et al*. (2009) nitrogen is an important element for increasing dry matter yields without affecting the essential oil content significantly. Oregano plants remove from the field 25-86 kg N ha^{-1} (Dordas, 2012). A basic application with a mixed-type fertilizer (e.g. 13-10-10) by the end of winter to early spring is satisfactory. After the first cutting, an additional fertilizer implementation may be needed. Apart from the macro-elements, sodium and secondarily copper might be also required. However, high levels of copper in the soil may be blamed for small, clorotic oregano leaves, with many stomata and few chloroplasts (Panou-Filotheou *et al*., 2001).

As oregano is rather tolerant to drought (Katsiotis and Chatzopoulou, 2010; Marques *et al*., 2012), it can be cultivated as rainfed crop. However, during particularly dry periods, one or two irrigation applications with about 40-50 mm per application may substantially increase yield without negatively affecting yield quality.

Weeds – Pests and diseases control

Weed control in a perennial crop such as oregano is necessary to keep the crop clean throughout the growing period. Especially in the year of establishment, weed control is very important especially by the end of winter-onset of spring.

As many winter or summer species infest oregano fields, tilling is therefore frequent, taking place at least two or three times a year (e.g. in autumn, spring and summer) (Marzi, 1996).

Harvest

The exact time of harvesting is determined on the yield quality basis, e.g. essential oil content upon flowering (usually mid-June to late August). Harvesting is normally carried out mechanically, whereas post-harvest processing is occasionally manual. The plants are cut 5-8 cm above ground. If the crop is irrigated, a second harvest may be possible; in this case, irrigation is needed immediately after the first harvest. Oregano is harvested at full blooming for essential oil production or at the beginning of flowering for herb production. Significant differences in yield and oil content have not been observed during the flowering period. Oil content in leaves is very low in the autumn (October) harvest. Highest yields are obtained in the second year, during the two cuts made in June-July and in October. Dry oregano yields may reach 1.5-3.0 t ha^{-1}, whereas the total duration of Greek oregano cultivation may exceed 10-11 years. A rough estimate of an average crop production (subject to further verification) indicates a value of 20 t ha^{-1} in a 4-year cultivated field. A full crop performance may be attainable after the first year of establishment. The harvest index is about 50-55% in the first harvest owing to the high incidence of stems and ca. 60-70% in the second mowing usually done in October. Finally, if oregano is intended for the market as fresh or frozen product, the plants can be harvested every 5-6 weeks. In this case, the upper plant parts are cut before flowering can be set.

Processing

The drying method plays major role for producing a high-quality product. During the drying process, drying temperatures are 30-40 °C should not be exceeded as they negatively affect the oil content (Marzi, 1996). The final product consists of a mixture of leaves and flowers, which account for about 40-60% of the aerial part of the dried plants. The relative volume by weight (specific gravity) of the fresh air portion is 80-120 kg m^{-1} (Katsiotis and Chatzopoulou, 2010). As direct exposure to sun can be rather dangerous, drying should take place in shade by spreading the harvested material in thin coats and applying ventilation of dry air. Another method is drying by heating. It is used on an industrial level where moisture is dropped by discharging hot air. An alternative form of artificial drying that is well used in the food industry for effective drying aromatic and medicinal plants is freeze drying known as lyophilization. The product is cooled and then dried by sublimation of ice in a vacuum. The advantage of this method is the stability of the plant material properties, but its substantial cost comprises a serious drawback.

Essential elements composition and oregano utilization

Elements composition

The essential oil content, as well as the ratio of carvacrol to thymol to the total oil amount is rather variable in the different Greek oregano populations. The very high values of essential oil yield (>7 ml/100 g dm) have been recorded on Crete and Amorgos islands, as well as in Gythion and Athos Peninsula. In some cases, the essential oil consists of a high carvacrol quantity, as in the South Peloponnese (more than 90% of the total oil), or in other cases thymol comprises the predominant phenol (Corfu Island). In these cases, carvacrol, i.e. the compound characterizing the oregano plant, is a minor constituent. The dominance of thymol suggests that these should belong to the group of plants used as thyme (Fig. 2, Table 1).

The essential oil content is much lower in autumn plants, ranging from 1.0 to 3.1% (ml/100 g dm), compared with those collected from the same areas in summer (4.8-8.2%). The most impressive difference is the increased amount of p-cymene in autumn: its amount ranges from 17.3 to 26.9% of the total oil in plants from South Peloponnese and Crete (instead of 4.0-9.5% found in the summer plants) to 37.1-51.3% of the oil in plants from Athos peninsula (instead of 12.0-12.2% in the summer) (Kokkini *et al*., 1996).

These results suggest that the essential oils of *O. vulgare* subsp. *hirtum* are characterized by stability, irrespective of harvesting time, with regard to (1) the high concentration of the sum of the four components involved in the phenolic biosynthetic pathway, and (2) the predominant phenol type. It should be noted that the two monoterpene hydrocarbons are very common constituents of all 'oregano' or 'thyme' type essential oils (Fig. 3) (Lawrence, 1984; Ravid and Putievsky, 1986; Kokkini, 1994).

Fig. 2. *Origanum vulgare* subsp. *hirtum* (from an experimental field at University of Thessaly)

Fig. 3. Essential oil yield, carvacrol (white bars) and thymol (black bars) contents (as percentages of the total oil) of *Origanum vulgare* subsp. *hirtum,* in different Greek localities (after Vokou *et al.*, 1993)

Table 1. Essential oil composition of 4 new oregano clones in Greece (Goliaris *et al*., 2002)

Components	Olympos	Vermio	Pilio	Athos
	% of total oil			
a-Thujene	0.07	0.81	0.12	0.52
a-Pinene	0.07	0.65	0.21	0.48
Camphene	0.06	0.15	0.05	0.09
Sabinene	0.50	0.80	1.12	0.66
Mycrene	0.13	1.70	0.40	1.09
a-Phellandrene	-	0.17	-	0.02
a-Terpinene	-	0.73	0.22	0.40
p-Cymene	0.74	4.25	4.05	8.10
Terpinolene	0.08	0.21	0.10	0.14
Limonene	-	0.17	0.08	0.16
Γ-Terpinene	0.55	2.43	0.32	0.63
Trans-sabinen-hydrate	0.02	0.51	0.31	0.46
Terpinen-4-ol	0.42	0.42	0.49	0.43
a-Terpineol	0.31	0.26	0.12	0.19
Thymol	0.32	0.28	0.04	0.55
Carvacrol	92.90	82.68	86.97	79.45
a-Cubenene	0.08	0.08	0.09	0.06
b-Caryophyllene	0.30	0.70	1.58	1.29
Trans b-Farnesene	0.11	0.12	0.09	0.14
a-Humulene	0.06	0.08	0.19	0.09
Muurolene	0.17	0.16	0.04	0.08
γ-Cadinene	0.24	0.08	0.60	0.70

Oregano utilization

Aromatic plants produce organic compounds that may be involved in the defense of plants against phytopathogenic insects, bacteria, fungi, and viruses. Most essential oils of the family Lamiaceae consist of monoterpenes and sesquiterpenes. Their activity is attributed to the content of carvacrol and thymol which are accompanied by p-cymene and c-terpinene that are the major constituents in oregano (Vokou *et al*., 1993).

Oregano is an important aromatic herb for its flavor and use as food (mixture of dry leaves and inflorescences) but also for its important medicinal uses. The essential oil of *Origanum vulgare* ssp. *hirtum* shows a high content of carvacrol, thymol, c-terpinene, and p-cymene, representing 73.7%, 92.8%, and 87.78% of the total oil, respectively (Sivropoulou *et al*., 1996; Lewinsohn *et al*., 2000; Bouchra *et al*., 2003; Baydar *et al*., 2004; Bozin *et al*., 2006).

Its activities include antioxidative properties in food (e.g. lard, sunflower oil) and in vivo and the inhibition of foodborne and human antibiotic-susceptible and antibiotic-resistant pathogenic bacteria, viruses, pathogenic fungi, parasites, insects in vitro and in human foods (e.g. apple juice, eggs, leafy greens, meat and poultry products, milk, oysters) and food animal feeds and wastes (Kirimer *et al*., 1995; Adam *et al*., 1998; Ozkan *et al*., 2003; Chami *et al*., 2005; Tajkarimi *et al*., 2010). Also, it covered inhibitions of microbial and fungal toxin production and the anti-inflammatory, analgesic, antiarthritic, antiallergic, anticarcinogenic (Conforti *et al*., 2008), antidiabetic, cardioprotective, gastroprotective, hepatoprotective, and neuroprotective properties of carvacrol as well as metabolic, synergistic, and mechanistic aspects (Bozin *et al*., 2006; Friedman, 2014).

Oregano is used as a seasoning food in cooking, and less as a beverage, which is referred to as excellent against coughing. For example, the essential oil of *O. hirtum* rich in carvacrol has been used as medicinal (Baricevic and Bartol, 2002), whereas the essential oil of *O. dubium* is used as antirheumatic (Arnold *et al*., 1993). Oregano has 12 times more an antioxidant action than orange, 30 times more than potato and 42 more than apple.

The therapeutic value of oregano was known already from ancient times. The ancient Greeks were drinking oregano tea in colic and also for external uses. The essential oil of oregano inhibits completely the mycelial growth of *Aspergillus niger* and *A. flaous* at 400 mg/ml, while *A. ochraceus* is inhibited at 600 mg/ml. Fungal spore germination is inhibited by 600 mg/ml of origanum oil and (with the exception of *A. ochraceus*) with 700 mg/ml of thyme oil (Paster *et al*., 1990). Under aerobic conditions, the essential oil of oregano (250 mg/ml) inhibited to some extent the growth of *Staphylococcus* aureus and *Salmonella typhimurium*. *Pseudomonas aeruginosa* was not affected by oregano oil at concentrations up to 500 mg/ml. The origanum oil is very effective against *Campylohacter jejuni* and *Clostridiurn sporogenes*. The essential oil of *O. vulgare* ssp. *hirtum* is extremely bactericidal at 1/4000 dilution and even at dilutions as high as 1/50000 caused a considerable decrease in bacterial growth rates. The same essential oil also exhibits high levels of cytotoxicity against four permanent animal cell lines including two derived from human cancers (Paster *et al*., 1990; Sivropoulou *et al*., 1996). Carvacrol causes a total obstructing in the development of colonies of fungus *Penicillium digitatum, Fusarium solani* var. *coeruleum* and *Botrytis cynerea* (Daferera *et al*., 2000; Kulisic, 2004; Chami *et al*., 2005).

The oil of *O. vulgare* shows a strong antibacterial action against Gram-negative bacteria (*Esherichia coli, Salmonella typhimurium, Proteus*, *Yersinia enterocolitica, Serratia marcenscens, Pseudomonas flourescens* and *Pseudomonas putida*), as well as positive, Gram-negative bacteria (*Micrococcus* sp., *Sarcina flava, Staphylococcus aureus, Bacillus licheniormis, Bacillus thuringiensis* and *Listeria innocua*) compared with the essential oils of other plants while their biosynthetic precursors c-terpinene and p-cymene were inactive (Pellequer *et al*., 1980; Gergis *et al*., 1990; Remmal *et al*., 1993; Sivropoulou *et al*., 1996; Marino *et al*., 2001). The essential oils of *O. vulgare (onites* and *hirtum)* show strong antibacterial action to the *Stenotrophomonas maltophilia* (Sarac *et al*., 2009). Also, the rich quantity in carvacrol of *Origanum vulgare* show the same strong activity against the bacteria *E. coli, S. aureus, Bacillus megaterium* and *Salomonella badar* (Remmal *et al*., 1993). The results of the study encourage the examination of the effectiveness of the essential oil of oregano against other pathogens, which are non-discriminatory and those that cause malignant diseases (Manohar *et al*., 2001).

Conclusions

Aromatic and medicinal plants have gained much popularity in European and global markets in the last decade. The yields of such plants and particularly of *Origanum vulgare* obtainable in Greece are of high quantity and best quality. Thus, the introduction of oregano in crop rotation systems in Greece should be seriously considered for the near future. It is believed that cultivation of oregano may play an important role for Greek agriculture by providing high added value also on degraded slopping lands in the semi-arid zone of the country. This should be the subject of future land evaluation studies of oregano versus conventional cropping systems in specific soil-climatic environments so that cost/benefit projections can be produced. Extensive cultivation of oregano might be proved to be highly beneficial also for the environment, with respect to biodiversity conservation and minimizing soil erosion, land degradation and desertification processes in many degraded lands of the semi-arid zone in Greece, where oregano cultivation might be proved to be ideal.

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