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Antimicrobial Activity of Essential Oils Extracted from Cultivated Oregano (*Origanum vulgare*), Sage (*Salvia officinalis*), Thyme (*Thymus vulgaris*) and Rosemary (*Rosmarinus officinalis*) against Clinical Isolates of *Escherichia coli*, *Klebsiella oxytoca*, *Klebsiella pneumonia* and *Listeria monocytogenes*

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Abstract

Oregano (*Origanum vulgare*), sage (*Salvia officinalis*), thyme (*Thymus vulgaris*) and rosemary (*Rosmarinus officinalis*) are aromatic plants with ornamental, culinary, and phototherapeutic use all over the world. In Europe, they are traditionally used in the southern countries, particularly in the Mediterranean region. The antimicrobial activities of the Essential Oils (EOs) derived from those plants have captured the attention of scientists as they could be used as alternatives to the increasing resistance of synthesized antibiotics against pathogen infections. Therefore, significant interest in the cultivation of various aromatic and medicinal plants is recorded during the last years. However, to gain a proper and marketable chemotype various factors during the cultivation should be considered as the variety, the geographical morphology, climatic conditions and farming. In this frame, we have studied the antimicrobial efficiency of the EOs from oregano, sage, thyme and rosemary cultivated under different conditions in a region of NE Greece in order to be proposed for profitable farming.

Keywords: *E. coli*; *K. oxytoca*; *K. pneumonia*; *Listeria monocytogenes*; Thyme; Oregano; Sage; Rosemary; Essential oils

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Introduction

During the last years, a significant problem has arisen due to the overuse and misuse of antibiotics; many pathogens are often resistant to common antibiotics. Among them *Escherichia coli, Klebsiella pneumoniae, Klebsiella oxytoca* and *Listeria monocytogenes* which are pathogenic microorganisms and can cause very severe illness [1-6]. These reports have shown that extracts from plants can be used to delay or inhibit the growth of pathogenic or spoilage microorganisms [7] and thus, may be the alternative natural way to fight the resistant pathogenic microorganisms.

The Mediterranean countries are the biggest producers of native aromatic plants [8] due to the ideal climatic conditions and geographical morphology. Greece is a country which produces hundreds of species of aromatic plants which are native [9]. Among them oregano, sage, thyme and rosemary widely used in Greek and global gastronomy, are endemic and are therefore suitable for cultivation under the climatic conditions in most parts of Greece. More specifically Oregano (*Origanum vulgare* subsp. *Hirtum*), which is a perennial plant of the genus Origanum in the family of Labiatae, indigenous plant of the Mediterranean region, spread to almost all the Mediterranean

countries (Europe and Africa), but also to the temperate zones of Asia and America. Oregano and essential oil of oregano are used in many sectors of modern industry as well as domestically. In Greek cuisine, oregano occupies a prominent position since it is used very much and mainly in grilled meat and salads. The food industries use the flavor of oregano in their products (potato chips, sauces, olive pastes, etc.). Oregano's EOs is also used in perfumery. Its main uses include perfuming spaces, in sports massage oils and in sauna [10]. In recent years, essential oil has also been used in medicine as a dietary supplement or as a booster of the immune system [11]. Dry oregano (stem, leaves, and flowers) has been shown to contain 2-8% of essential oil [12]. The main ingredients of the essential oil of oregano are carvacrol, thymol, γ-terpine and p-cymen [13]. Content ranges up to 80% for carvacrol and up to 15% for thymol largely determines the quality of the plant itself, it's essential oils, the antimicrobial activity and more import its commercial value [14,15]. However, the natural oil of oregano contains more than 30 components which also contribute to the antibacterial activity of the oil [16-18].

Thyme (*Thymus vulgaris* L.) is another perennial herb of the Thymus genus of Thymus and belongs to the family of *Labiateae*. Thyme contains 1-2% of essential oil, which varies from the type of thyme, the area where it is cultivated and the harvest season [19-22]. The essential ingredients of the thyme oil are thymol, carvacrol, linanol, geraniol, α-terpineol, γ-terpinene and p-cymene. It also includes flavonoids (luteolin derivatives), pentacyclic triterpenes (uric acid, oleic acid), tannins and caffeic acid [23]. Thymol is the key ingredient largely determines the quality of thyme and its essential oil. The content ranges from 50% to 70% for thymol substances which among other, possesses antiseptic properties and strong antimicrobial activity [24-26]. Although it is the major component of the oil, the natural thyme oil contains more than 30 components [16,18].

Sage (*Salvia officinalis*) is another perennial aromatic plant of the genus *Salvia* and belongs to the family of *Labiateae*. It is a native plant of southern Europe and the countries of the Mediterranean. In the genus *Salvia* there are approximately 900 species [27-30]. The essential oil is characterized by two major classes of secondary metabolites: terpenoids and phenols. The main components of the essential oil are: α-thuyone, β-thuyone, camphor, 1,8-cineol (eucalyptol), camphene, α-pinene, β-pinene [24]. The commercial value of the essential oil is determined by the percentages of α- and β-thuyone. According to Novak et al., (2006) [31] these percentages should be more than 30% and camphor more than 20%.

Rosemary (*Rosmarinus officinalis*) is a perennial plant of the genus the *Rosmarinus* and belongs to the family of *Labiateae* with often a height of 2 m. The origins of rosemary are from the Mediterranean regions but today it is cultivated in almost all Europe and America. Its content of essential oil ranges from 1.0-2.5% while the chemical structure includes components of phenolic (rosmarinic, chlorogenic) and caffeic acids, linoleol and additionally of flavonoids with strong antioxidant properties [24,32]. The anti-inflammatory properties of the herb are explained by the presence of terpenoids such as rosmanic and uricolic acid, rosmanol and carnosol. The main ingredients found in abundance in its oil are a-pinene, 1,8-cineeol, camphor, camphene, verbenone and borneol [33].

Although some of the key components of EOs are characterized by strong antibacterial properties and their mode of action resembles to the mode of action of antibiotics [34], it is unlikely that they will be used soon in therapeutics or as food preservatives mainly because of the limited number of bacterial strains tested and the differences in their susceptibilities. Thus, the application of such compounds as either therapeutics or as food preservatives should be tested against a larger number of strains and different bacterial species to determine their usefulness. As most of the published reports were concerned with the antibacterial properties of EOs against common food pathogens, in this study we focused on four species of clinical origin with proven resistance to antibiotics. Additionally, it was our aim to study if the antimicrobial action of EOs was differentiated by the farming method (water availability, fertilization) of aromatic plants in order to promote their selection as a cost-effective yet profitable cultivation.

Materials and Methods

Plant material

Seedlings of oregano (*Origanum vulgare* subsp. hirtum), sage (*Salvia officinalis*), thyme (*Thymus vulgaris*) and Rosemary (*Rosemarinus officinalis*) per species were initially supplied from a certified institute (DIOnet, Thessaloniki, Greece) to ensure that all plants are of the same genotype. Cultivation was set up at an experimental field located in NE Greece in May 2016. The cultivation consisted of two separated experimental blocks per species with the first block been properly taking care of frequent irrigation and fertilization and the second block left under drought and without any further effort. Each block had four plant species each species had four rows and each row had twenty plants, totally eighty plants. There were two harvests in the first and second year after planting during the months June-July and September-October at full blooming of plants. Aerial parts were air dried in well-ventilated dark rooms for 20 days before EOs extraction. A voucher specimen of each plant species is deposited in the herbarium of the Department of Agricultural Development with a reference number from DAD-LM-01 to DAD-LM-04.

Isolation of essential oils

Essential Oil concentration of leaves and inflorescences were isolated from the air-dried material by a Clevenger apparatus using 220 g of ground plant samples diluted in 1,500 mL deionized water each time [7,35,36]. The mixture was boiled for 3 h and the volume of the EOs produced was measured and expressed as mL/100 g dry weight of the plant. Dehydration was achieved by using anhydrous magnesium sulfate (Sigma-Aldrich). All EOs were stored in amber glass vials at -30°C until use.

Bacterial strains

The antibacterial assay was performed against Gram positive and negative bacteria. Specifically, against seven strains of *Klebsiella oxytoca*, sixteen strains of *Klebsiella pneumonia*, twenty seven strains of *Escherichia coli* and five strains of *Listeria monocytogenes*. All strains were collected, verified (Vitek 2, Biomerieux, France) and tested for resistance against nine antibiotics from the local University hospital (Table 1). Strains were cultured in suitable agar plates; Tryptone bile x-glucuronide medium (TBX) agar (Oxoid Ltd., UK) for *Escherichia coli*, M-endo agar (Sigma-Aldrich) for *Klebsiella* spp and ALOA agar (Agar Listeria Ottaviani & Agosti) for *Listeria monocytogenes*. After 24 h incubation at 37°C, isolated colonies were frozen at -20°C in Tryptic Soy Broth (Oxoid Ltd, UK) supplemented with 15% (v/v) glycerol until used. As reference strains, *E. coli* NCTC 10410, *K. oxytoca* NCTC 49131, *K. pneumoniae* sub sp. *pneumoniae*, NCTC 11228 and *Listeria monocytogenes* ATCC 7644 have been used (Table 1).

Table 1: Susceptibility profile (%) of clinical pathogens.

Strain	Antibiotic (susceptible breaking point, mg/L)[a]								
	AK (≤16)	AM (≤8)	AUG (≤8/4)	CAZ (≤4)	CPE (≤8)	CPT (≤0.5)	IMP (≤1)	MER (≤1)	P/T (≤16/4)
E. coli, (*n*=27)	100	41.4	65.5	100	93	79.3	100	100	79.3
K. oxytoca, (*n*=7)	71.4	0	57.1	57.1	100	57.1	100	100	57.1
K. pneumoniae, (*n*=16)	81.6	0	65.8	60.5	65.8	57.8	65.8	65.8	68.4
L. monocytogenes, (n=5)	nt	1	nt	nt	nt	nt	nt	0.25	nt

[a]CLSI breaking points (CLSI, 2011)[37]. AK: Amikacin; AM: Ampicillin; AUG: Amoxicillin/clavulanic acid; CAZ: Ceftazidime; CPE: Cefepime; CPT: Ceftaroline; IMP: Imipenem; MER: Meropenem; P/T: Piperacillin/Tazobactam; nt: not-tested

Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration (MIC) of EOs was determined by the broth micro dilution method according to Clinical Laboratory Standards Institute (CLSI, 2011) [37-39]. All strains were cultured in Muller Hinton agar (Oxoid, UK) at 37°C for 16 h prior to MIC determination. An inoculum density of $1.5x10^8$ cfu/mL equivalent to 0.5 McFarland units of each of the test organisms was prepared in sterile saline (0.84% NaCl). One hundred microliters (100 μL) of double strength Muller Hinton Broth (MHB) containing 5% dimethyl sulfoxide (DMSO) was dispensed into wells of 96-well micro plates. In the first column of wells, EOs were added in amounts to achieve an initial concentration of 1024 mg/L and then serially diluted (by two-fold) across the plate (cells 1 to 10) to a final concentration of 1 mg/L. One hundred microliters (100 μL) of bacteria suspension were finally added to each well and the plates were incubated at 37°C for 24 h. Columns 11 and 12 of the micro plate were used as positive (with bacteria and without EOs) and negative (without bacteria inoculum) controls. When necessary, the experiment was repeated with decreased initial concentration of the EOs in order for the MIC of each strain to be determined. The assay for each of the pathogens was repeated three times. Growth of bacterial cells in each of the wells was verified by the color formed after the addition of 20 mL of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) prepared at a concentration of 5 mg/mL in PBS, and additional incubation for 30 min.

Statistical analysis

Average and standard deviations of MIC values were estimated. Mean MIC values (mg/L) between different groups were compared by using Analysis of Variance (ANOVA) with Fisher's Least Significant Difference (LSD) procedure at a significance differences level of 0.05. All statistical analyses were performed with SPSS v17 (SPSS Inc., USA).

Results and Discussion

The antimicrobial activity of the EOs collected from *Origanum vulgare*, *Salvia officinalis*, *Thymus vulgaris* and *Rosemarinus Officinalis* to the above pathogenic microorganisms is shown in Table 2-5.

Oregano

The mean MIC for the 26 strains of *E.coli* ranged from 11.6-231.7 mg/L and from 14.7-197.4 mg/L for irrigated and non-irrigated cultivations respectively (Table 2). For both treatments, the lowest value of mic, and thus the most active essential oil against *E. coli*, was in the first October. Correspondingly to the first bloom in June for both years, for both treatments, the oil of the October harvests appears to be more drastic. As for the comparison between the two treatments, there were no statistically significant differences for the same harvesting periods. The results show a large deviation between the minimum and maximum of MIC values, but are significantly more drastic than MIC values in literature such as value 200-250 mg/L [40-42]. Similarly, other studies with MIC values of 0.5 mg/L [43-45] and 31.25-40 mg/L (Şahin et al., 2004; Peñalver et al., 2005) [46,47] may also be justified by extremely different chemotypes in plants. For *K. oxytoca* the minimum inhibitory concentration of the oregano essential oil against 8 strains was tested. The average MIC for the strains of *Kl. oxytoca* ranged from 1-88.4 mg/L and from 2-88.4 mg/L for irrigated and non-irrigated cultivations, respectively. For both treatments, the lowest value of mic, and therefore the most active essential oil against *K. oxytoca*, was obtained in the first June, (mic 1-2 mg/L respectively) and the highest mean value of mic in the second June (88 mg/L) in both treatments. As for the comparison between the two treatments, there were not observed statistically significant differences. The mean mic for the 16 strains of *K. pneumoniae* ranged from 12-98.3 mg/L and from 14.9-70.2 mg/L for irrigated and non-irrigated cultivations, respectively. For both treatments, the lowest mean mic, hence the most active essential oil against *K. pneumoniae*, was at the second bloom of the plant in the second October for the irrigated and the first bloom in the second June for dry cultivation. In both treatments in the first year for both harvests there were high MIC values without statistically significant differences between them, while in the second year MIC values were much lower, which means the more the plant matured the more active essential oil had against *K. pneumoniae*. With regard to the comparison between the two treatments, the essential oil of dry oregano appears to be more effective comparing the same harvesting periods. In the studies of other researchers, various species of *Klebsiella* spp. appear to be either susceptible to oregano essential oil with MIC values of 0.5 mg/L [43], moderately susceptible to MIC close to 250 mg/L [41] or extremely resistant and not affected by the essential oil of oregano [46]. The mean mic for the five strains of *L. monocytogenes* ranged from 0.5-83.2 mg/L and from 0.2-96 mg/L for irrigated and non-irrigated cultivations, respectively. Table 2 shows that for both harvests except for the first Octobers harvest, the oregano essential oil was very drastic with mean mic 0.5 mg/L. In literature, species of *L. monocytogenes* appear either to be susceptible to essential oil with MIC values of 0.0625 mg/L [47] or more resistant to 100 mg/L [42] (Table 2).

Rosemary

For *E. coli* the minimum inhibitory concentration of rosemary essential oil was tested against 26 strains (Table 3). The mean MIC for the strains of *E. coli* ranged from 212-420.2 mg/L and from 249.2-394.2 mg/L for the irrigated and dry cultivations respectively (Table 3). MIC for both treatments was very high. The lowest value of MIC, therefore the most active essential oil against *E. coli*, was detected in the first year, while in second year there was a 30% reduction in activity for both crops. As for the comparison between the two treatments, there were not many different ones, although the

Table 2: Minimum Inhibitory Concentration (MIC,mg/L) of essential oils of oregano against clinically isolated pathogens for two different treatments.

Organism	Value	MIC (*Origanum vulgare*)							
		Irrigated				Non-irrigated			
		1st June	1st Oct	2nd June	2nd Oct	1st June	1st Oct	2nd June	2nd Oct
E. coli	N	26	26	26	26	26	26	26	26
	Mean	231.7[a]	11.6[b]	53.2[c]	41.8[c]	197.4[a]	14.7[b]	55.3[c]	43.3[c]
	SD	141.8	13.2	51.1	79.0	135.9	18.7	57.3	72.7
	MIN	8	0.0625	0.44	0.85	7	0.1	0.875	1
	MAX	512	56	192	256	512	64	224	224
K. oxytoca	N	8	8	8	8	8	8	8	8
	Mean	1.0[a]	5.8[a]	88.4[b]	8.3[a]	2.0[a]	6.2[a]	88.4[b]	11.0[a]
	SD	0.8	10.9	77.5	10.7	2.5	9.3	77.4	18.8
	MIN	0.078	0.15	0.87	2	0.0625	0.14	1.75	1.75
	MAX	2	32	224	32	7	28	224	56
K. pneumoniae	N	16	16	16	16	16	16	16	16
	Mean	97.4[a]	98.3[a]	23.5[b]	12.0[b]	52.7[a]	70.2[a]	14.9[b]	18.7[b]
	SD	105.8	105.8	63.9	9.4	47.1	69.7	33.0	26.9
	MIN	0.0625	0.78	0.125	1.75	3.5	3.5	0.109	0.125
	MAX	256	256	256	32	128	256	128	112
L. monocytogenes	N	5	5	5	5	5	5	5	5
	Mean	0.5[a]	83.2[b]	0.5[a]	0.5[a]	0.5[a]	96.0[b]	0.5[a]	0.2[a]
	SD	0.7	42.9	0.7	0.7	0.7	45.3	0.7	0.1
	MIN	0.125	32	0.125	0.125	0.125	32	0.0625	0.125
	MAX	1.75	128	1.75	1.75	1.75	128	1.75	0.25

Same superscript letters indicate non-significant differences (p>0.05) in column groups.

Table 3: Minimum inhibitory concentration (MIC,mg/L) of essential oils of rosemary against clinically isolated pathogens for two different treatments**.**

Strain	Value	MIC (*Rosemarinus officinalis*)							
		Irrigated				Non-irrigated			
		1st June	1st Oct	2nd June	2nd Oct	1st June	1st Oct	2nd June	2nd Oct
E. coli	N	26	26	26	26	26	26	26	26
	Mean	212.0[a]	297.8[a]	412.3[b]	420.2[b]	251.7[a]	249.2[a]	394.2[b]	378.5[b]
	SD	140.6	236.3	193.6	303.4	269.8	268.0	196.2	269.9
	MIN	40	112	64	28	32	56	56	56
	MAX	512	896	896	1024	896	1024	896	896
K. oxytoca	N	8	8	8	8	8	8	8	8
	Mean	190.0[a]	118.0[a]	193.0[a]	320.0[b]	210.0[a]	113.5[a]	179.0[a]	274.0[b]
	SD	138.8	136.2	186.9	313.5	168.5	137.1	128.1	287.2
	MIN	112	32	56	128	56	28	56	112
	MAX	512	448	640	1024	448	448	448	896
K. pneumoniae	N	16	16	16	16	16	16	16	16
	Mean	258.0[a]	247.8[a]	241.0[a]	390.0[b]	230.0[a]	244.6[a]	237.5[a]	358.8[b]
	SD	215.0	210.1	126.9	281.4	219.6	235.6	210.2	262.2
	MIN	64	28	112	64	56	26	56	28
	MAX	896	896	512	1024	896	896	896	896
L. monocytogenes	N	5	5	5	5	5	5	5	5
	Mean	83.2[a]	160.0[b]	153.6[b]	128.0[a]	76.8[a]	166.4[b]	128.0[a]	128.0[a]
	SD	42.9	96.0	57.2	0.0	28.6	85.9	78.4	117.6
	MIN	32	32	128	128	64	64	64	32
	MAX	256	256	256	128	256	256	256	256

Same superscript letters indicate non-significant differences (p>0.05) in column groups.

essential oil of the dry crop against this microorganism appears to be slightly less active. For *K. oxytoca* the mean MIC ranged from 118-320 mg/L and from 113.5-274 mg/L for irrigated and dry cultivations, respectively. For treatments, the lowest MIC value, and thus the most drastic essential oil against *K. oxytoca*, was detected in the first October, the second bloom of the plant and the highest MIC in the second October. In both harvests in June, MIC was at the same level. As for the comparison between the two treatments, there were no statistically significant differences comparing the same harvesting periods. For *K.pneumoniae* the minimum inhibitory concentration of rosemary essential oil was tested against 16 strains. The mean MIC ranged from 241-390 mg/L and from 230-358.8 mg/L for irrigated and non-irrigated cultivations, respectively. All are considered quite high values or low activity. For all harvests for both crops, MIC values were close to around 240 mg/L except for the October harvest where we had an increase of MIC up to 30%. Consequently, the more the plant matured the less active was the essential oil against microorganisms such as *K.pneumoniae*. In both treatments, there was similar activity with the essential oil of the dry crop a little more active. For *L. monocytogenes* the mean MIC for all strains ranged from 83.2-160 mg/L and from 76.8-166.4 mg/L for irrigated and dry cultivations, respectively. From it appears that for both treatments the rosemary essential oil was more drastic in the first harvest of the first year, in June, in October of the same year the values of MIC were increased by 50%. In general we can say the more the plant matured, the less active its essential oil against *L. monocytogenes* was. For this reason it is possible to use essential oil from young plants for antimicrobial action. For both treatments, no essential oil showed any advantage over the strains of *L. monocytogenes*. In comparison with the literature, Ivanovic et al. (2012) [48] reported MIC values of rosemary essential oil over 2.560 mg/L for *E. coli*. Santurio (2011) [49] reported values above 6400 mg/L. However, other researchers found MIC values very low from 10-20 mg/L (Yesil-Celiktas et al., 2007, de Medeiros Barbosa et al.,2016), 0.3 mg/L [44,50] (Jiang et al., 2011) [51] at different harvest periods of rosemary against *E. coli*. Pesavento (2015) [47] found average MIC of only 2 mg/L against *L. monocytogenes* and De Azeredo et al. (2011) 20 mg/L [52] Kazemi (2012) [53] reports MIC values of rosemary essential oil against *K. oxytoca* and *K. pneumoniae* only 3 mg/L. The MIC of rosemary was 5mg/mL against *L. monocytogenes* and *E. coli* and 10mg/mL for Stojiljkovic et al. (2018) [54]. Also reported in the literature are MIC values from 125 mg/L [55] to over 1000 mg /L [56] of rosemary essential oil against *K.pneumoniae* strains (Table 3).

Thyme

For *E.coli* the minimum inhibitory concentration of thyme essential oil was tested against 26 strains (Table 4). The mean MIC strains of *E. coli* ranged from 24.6 to 44.8 mg/L and from 29.1 to 41.4 mg/L for irrigated and non-irrigated cultivations respectively (Table 4). MIC values were low, meaning that thyme essential oil was quite active against *E. coli*. For both treatments, the lowest value of MIC, and thus the most active essential oil against *E. coli*, was in the June harvests for both years. As for the comparison between the two treatments, there were no significant differences between them. Comparing the same harvesting periods, the essential oil of irrigated crops appears to be a bit more active in the first year, while in second year the essential oil of non -irrigated cultivation appears to be slightly more active. The mean MIC for the 8 strains of *K. oxytoca* ranged from 8.1 to 121 mg/L and from 4.6 to 156.9 mg/L for irrigated and dry cultivations respectively. For both treatments, the lowest MIC value, therefore the most drastic essential oil for *K. oxytoca*, was in the first June and the high MIC was in second June. As for the comparison between the two treatments, there were no statistically significant differences (Table 4). For *K. pneumoniae* the minimum inhibitory concentration of thyme essential oil was tested against 16 strains. The mean MIC for the strains of *K. pneumoniae* ranged from 11.4-33.3 mg/L and from 8.3-33 mg/L for irrigated and dry cultivations, respectively. For both treatments, the lowest value of MIC, and thus the most active essential oil against *K. pneumoniae*, was in the first June, the least active being in the second October , it seems that as the mature plant matured, the oil against the strains of *K. pneumoniae* is active as the MIC values have been tripled. Of the two treatments, neither seemed to have a clear precedence. These results were of the lowest compared to other published studies that used micro-dilution as a selection method for the antimicrobial assay. Burt and Reinders (2003) [57] reported MIC 625 mg/L for E. coli, Al-Bayati (2008) [58] reported values of 62.5 mg/L against *E. coli* and 500 mg/L for *K. pneumoniae*. Pei et al., (2009) [59] found values of 400 mg/L in their study and Costa et al., (2009) [60] reported values of 250 mg/L for the two species. However, some of the first studies from Greece also reported an increased antimicrobial effect of thyme essential oil with MIC values between 0.28 and 3.35 mg/L for *E. coli* and 0.72 mg/L for *K. pneumonia* [61]. The mean MIC for the five strains of *L. monocytogenes* ranged from 0.3 to 77.6 mg/L and from 0.2 to 64 mg/L for irrigated and dry cultivations respectively. From Table 4 it appears that for both treatments and for all harvests except for the harvests of first Octobers the thyme essential oil was active with MIC at 0.3 mg/L. Similar results for the antimicrobial activity of thyme essential oil against *L. monocytogenes* were Smith-Palmer et al. (1998) [62] with value 0.3 mg/L, Pesavento et al., (2015) [47] with 0.25 mg/L and Rota et al., (2008) [63] with MIC 0.2 mg/L (Table 4).

Sage

For *E. coli* the minimum inhibitory concentration of sage essential oil was tested against 26 strains the mean MIC ranged from 423.7-537.8 mg/L and 383.7-532.9 mg/L for irrigated and dry cultivations respectively (Table 5). The MIC for both treatments was quite large. On two treatments, the EOS of sage was more drastic when it was collected in the first June for irrigated cultivation and in the second October for dry cultivation. For *K. oxytoca* the minimum inhibitory concentration of sage essential oil against 8 strains was tested. The mean MIC for strains ranged from 153-294 mg/L and from 140-250 mg/L for irrigated and dry cultivations respectively (Table 5). For both treatments, the lowest value MIC and therefore the most active essential oil against *K. oxytoca* was collected in the first June in the first flowering plant and essential oil with the highest MIC was in the first October and the second June. For *K. pneumoniae* the minimum inhibitory concentration of the essential oil of rosemary against 16 strains with a mean value MIC of 237.3-424 mg/L was checked , and from 273 to 344 mg/L for the irrigated and non-irrigated cultivation respectively. For all harvests for both years MIC values were very high. *L. monocytogenes* was controlled by the minimum inhibitory concentration of the essential oil of sage against 5 strains averaging the MIC of 16.8-204.8 mg/L and 15.2-460.8 mg/L for the irrigated and non- irrigated cultivation respectively. Table 5 shows that for both treatments the essential oil of sage was more active in the June harvests in the first bloom, whereas in the October harvests had doubled value of MIC. Regarding the antimicrobial activity of the essential oil of sage, we did not notice any remarkable antibacterial activity since the MIC values recorded against all pathogens were

Table 4: Minimum inhibitory concentration (MIC,mg/L) of essential oils of thyme against clinically isolated pathogens for two different treatments.

Organism	Value	MIC (*Thymus vulgaris*)							
		Irrigated				Non-irrigated			
		1st June	1st Oct	2nd June	2nd Oct	1st June	1st Oct	2nd June	2nd Oct
E. coli	N	26	26	26	26	26	26	26	26
	Mean	24.6[a]	34.1[a]	29.8[a]	44.8[a]	35.0[a]	41.2[a]	29.1[a]	41.4[a]
	SD	37.2	36.4	67.0	76.2	60.5	38.6	59.8	72.8
	MIN	2	0.43	1	1.75	1.75	0.15	3.5	1.75
	MAX	128	160	256	256	256	144	224	256
K. oxytoca	N	8	8	8	8	8	8	8	8
	Mean	8.1[a]	14.6[a]	121.0[b]	23.0[c]	4.6[a]	15.8[a]	156.9[b]	22.2[c]
	SD	11.2	21.8	67.0	42.7	7.1	21.4	131.3	36.5
	MIN	0.0625	0.22	8	4	0.17	0.25	7	1.75
	MAX	32	64	256	128	16	64	448	112
K. pneumoniae	N	16	16	16	16	16	16	16	16
	Mean	11.4[a]	11.6[a]	14.8[a]	33.3[b]	8.3[a]	19.4[a]	14.4[a]	33.0[b]
	SD	12.4	13.2	27.0	42.3	10.8	39.5	27.1	38.7
	MIN	0.0625	0.21	0.25	1	0.025	0.125	0.125	1
	MAX	32	32	112	128	28	156.3	112	112
L. monocytogenes	N	5	5	5	5	5	5	5	5
	Mean	0.3[a]	77.6[b]	0.3[a]	0.3[a]	0.3[a]	64.0[b]	0.3[a]	0.3[a]
	SD	0.0	30.4	0.0	0.0	0.0	0.0	0.0	0.0
	MIN	0.25	64	0.25	0.25	0.25	64	0.25	0.25
	MAX	0.25	132	0.25	0.25	0.25	64	0.25	0.25

Same superscript letters indicate non-significant differences (p>0.05) in column groups.

Table 5: Minimum Inhibitory Concentration (MIC,mg/L) of essential oils of Sage against clinically isolated pathogens for two different treatments.

Organism	Value	MIC (*Salvia officinalis*)							
		Irrigated				Non-irrigated			
		1st June	1st Oct	2nd June	2nd Oct	1st June	1st Oct	2nd June	2nd Oct
E. coli	N	26	26	26	26	26	26	26	26
	Mean	423.7[a]	536.0[a]	537.8[a]	521.5[a]	431.1[a]	532.9[a]	504.6[a]	383.7[a]
	SD	242.0	263.4	224.3	321.9	245.2	294.9	211.9	295.5
	MIN	56	112	224	64	56	112	128	56
	MAX	1024	1024	1024	1128	896	1024	896	896
K. oxytoca	N	8	8	8	8	8	8	8	8
	Mean	153.0[a]	294.0[b]	292.0[b]	210.0[b]	140.0[a]	235.0[b]	250.0[b]	199.5[b]
	SD	121.5	252.5	141.5	285.4	139.6	275.6	92.9	287.7
	MIN	56	80	128	32	28	56	112	28
	MAX	448	896	512	896	448	896	448	896
K. pneumoniae	N	16	16	16	16	16	16	16	16
	Mean	353.3[a]	237.3[b]	322.0[a]	424.0[a]	315.9[a]	273.0[b]	344.0[a]	334.0[a]
	SD	307.6	249.2	125.0	348.3	325.4	292.4	139.5	296.4
	MIN	16	4	224	16	7	0.75	32	4
	MAX	896	1024	512	1024	896	896	512	896
L. monocytogenes	N	5	5	5	5	5	5	5	5
	Mean	16.8[a]	204.8[b]	16.8[a]	83.2[c]	15.2[a]	460.8[b]	15.2[a]	70.4[c]
	SD	10.0	70.1	10.0	42.9	10.7	114.5	10.7	35.1
	MIN	4	128	4	32	4	256	4	32
	MAX	32	256	32	128	32	512	32	128

Same superscript letters indicate non-significant differences (p>0.05) in column groups.

above 150 mg/L to 537.8 mg/L. And in the literature, the sage essential oil does not appear rude to the specific micro-organisms. Delamare et al., (2007) [64] reported that in sage had MIC values against *E. coli* between 5000 and 10000 mg/L and 100 mg/L against *K. oxytoca*. Ivanovic et al., (2012) [47] reported values above 2.560 mg/L for *E. coli*. Generalić et al., (2012) [65] found MIC values of 550-990 mg/L for *E. coli*. Miguel et al., (2011) [66] reported a very weak antibacterial activity of sage essential oil despite the different method used in their assay. Similarly, no antibacterial activity of sage essential oil against *E. coli* strains was observed at concentrations of 1000 mg/L or even as high as over 8000 mg/L for both *E. coli* and *K. pneumoniae*. However, much lower values close to 20 mg/L have also been reported for *K. pneumoniae* by Hammer et al., (1999) [67] and Wijesundara, N. M., & Rupasinghe, H. V. (2018) [47]. With respect to *L. monocytogenes*, Mazzarrino et al., (2015) [68] with MIC of higher than 40 mL/L give similar results, and there are fewer studies which clearly indicate lower inhibitory concentrations, such as the study by Soković et al. (2007) with 7 mg/L [69] (Table 5).

Conclusions

From the study of the antimicrobial action against the four species of microorganisms, the most effective essential oil was that of oregano, then thyme, rosemary and finally sage. More resistant micro-organisms were the gram-negative and especially *E. coli*, whereas *L. monocytogenes*, which is a gram-positive micro-organism, was more susceptible. There was no particular difference in the antimicrobial effect of essential oils isolated from two different treatments of plants (irrigated and fertilized *vs.* non-irrigated, non-fertilized). Despite other differences observed between irrigated and non-irrigated aromatic and medicinal plants, as for example in EOs, it seems that cultivated plants possess an equal yet species-dependent antimicrobial efficiency as the wild ones, which gives them commercial opportunities.

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