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Antimicrobial Activity of the Essential Oils of *Thymus vulgaris* L. Measured Using a Bioimpedometric Method

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ABSTRACT

The essential oils obtained from *Thymus vulgaris* L. harvested at four ontogenetic stages were evaluated for their biological activity and chemical composition. The thyme essential oils were tested for their inhibitory effects against nine strains of gram-negative bacteria and six strains of gram-positive bacteria. The bioimpedance method was chosen for studying the antibacterial activity of the essential oils and the parameter chosen for defining and quantifying the antibacterial activity of the essential oils was the detection time. The plate counting technique was used to study the inhibitory effect by direct contact. All the thyme essential oils examined had a significant bacteriostatic activity against the microorganisms tested. This activity was more marked against the gram-positive bacteria. The oil from thyme in full flower was the most effective at stopping the growth of the microbial species examined. The oils tested were also shown to have good antibacterial activity by direct contact, which appeared to be more marked against the gram-negative bacteria. Only a few of the species were capable of recovering at least 50% of their metabolic function after contact with the inhibitor, while most of the strains were shown to have been inactivated almost completely. *Escherichia coli* O157:H7 was the most sensitive species, given that after contact with even the lowest concentration of oil cells could not be recovered.

The essential oils, compounds extracted from various types of plants and used for preserving foods and drinks, have an inhibitory effect on the growth of microorganisms. Early work by Walton et al. *(31)* demonstrated that the distilled extract of garlic had bactericidal and bacteriostatic effects. Garlic (*Allium sativum*) has been used medicinally for centuries, as cited by Pliny, Virgil, and Hippocrates *(8)*.

Other parts of plants or their extracts, including garlic, onion, cinnamon, nutmeg, curry, mustard, black pepper, thyme, oregano, sage, rosemary, Jamaican pepper, aniseed, basil, paprika, turmeric, bay, cardamom, cassia, Cayenne pepper, celery, chives, clover, coriander, dill, ginger, savory, and marjoram, used as spices or aromatic herbs to give special aromas or flavors to foods are also known to have antimicrobial properties *(2, 4, 5, 10, 13–15, 17, 18, 20, 22, 24, 26, 29, 33, 34)*. Naturally, their activity depends on the type, composition, and concentration of the spice or the essential oils, the type and concentration of the microbial species, the composition of the substrate, the processing treatments, and the storage conditions *(13, 26)*.

El-Khateib and Abd El-Rahman *(12)* demonstrated that 4% of garlic used as a spice in sausages and beef hamburgers was a bacteriostat, while 5% led to a reduction in the bacterial load of an order of magnitude within the first 4 days. Ajoene, one of the six fractions extracted from garlic, at concentrations <20 μg/ml inhibits the growth of *Aspergillus niger* and *Candida albicans (32). Salmonella* Typhimurium, *Staphylococcus aureus*, and *Vibrio parahaemolyticus* are inhibited by the presence of powdered thyme, mint and bay leaves, and their alcoholic extracts *(1)*; the growth of these microorganisms is inhibited by the addition of 1,000, 5,000, and 6,000 ppm of thyme, bay leaves, and mint, respectively.

Several essential oils, including the oils from cedar, eucalyptus, and camomile, have been demonstrated to be effective particularly at inhibiting the germination and vegetative growth of spores of *Bacillus cereus* and *Clostridium botulinum (7)*.

Thymol, anethole, menthol, and other essential oils from Jamaican pepper, cinnamon, clove, garlic, oregano, sage, and thyme can inhibit the growth of food pathogens and yeasts found in foods *(8, 18, 20, 21)*. These essential oils can be used to prevent the growth of molds in foods and their contamination with aflatoxins *(20)*. The essential oil in oregano has been demonstrated to be particularly efficient at inhibiting the growth of *A. niger*, *Fusarium oxysporum*, and *Penicillium* spp. *(9)*.

Recently, selected spices and their essential oils have been studied with the aim of inhibiting the growth or contamination of *Listeria monocytogenes* in foods. Jamaican pepper, cloves, cumin, garlic powder, cinnamon, oregano, sage, thyme, paprika, red and black pepper, and rosemary, in addition to the essential oils of fir and pine trees, have given good results in terms of their capability of reducing the number of these microorganisms *(3, 6, 17, 23, 26, 30)*.

The reduction of the microbial population depends on the concentration of the spice or the essential oil; high concentrations of spice or essential oil have antibacterial and bacteriostatic effects and can completely inhibit the growth

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TABLE 1. *Detection times in the presence of various concentrations of* Thymus vulgaris *TAF essential oils*[a]

	Control	50 ppm	100 ppm	200 ppm	400 ppm
Escherichia coli	3.4	4.6 F	5.0 H	7.9 GH	9.2 HI
E. coli O157:H7	4.3	5.5 E	11.5 DE	30.6 A	50.7 A
Proteus mirabilis	2.8	4.1 G	6.8 G	7.7 H	9.5 HI
P. vulgaris	2.7	3.9 G	5.0 H	9.2 G	16.2 F
Salmonella Typhimurium	3.0	3.2 I	3.3 I	3.5 I	12.5 G
Serratia marcescens	3.0	3.2 I	3.4 I	3.5 I	7.3 I
Yersinia enterocolitica	6.0	7.1 D	18.3 A	22.9 B	27.5 D
Pseudomonas fluorescens	10.3	13.4 A	14.3 C	15.2 D	23.9 E
P. putida	8.5	10.1 B	11.8 D	12.2 EF	18.0 F
Micrococcus spp.	5.4	8.3 C	9.7 F	13.1 E	31.5 C
Sarcina flava	3.8	8.6 BC	16.9 B	21.8 B	28.5 D
Staphylococcus aureus	3.1	3.6 H	4.2 HI	4.2 I	10.1 GH
Bacillus licheniformis	6.7	15.3 A	17.2 AB	18.6 C	27.3 D
B. thuringiensis	3.5	9.8 BC	18.3 A	22.0 B	46.3 B
Listeria innocua	5.1	8.4 C	10.4 EF	11.3 F	27.5 D

[a] Data in the column followed by different letters are significantly different at $P < 0.01$ according to Tukey's HSD test.

of microorganisms. Essential oils are classified as generally recognized as safe; however, high concentrations of spices or essential oils can give undesirable aromas and flavors when the doses used exceed organoleptically acceptable levels; for this reason the use of essential oils or spices is often limited.

The aim of this work was to study the antibacterial activity and chemical composition of four essential oils from *Thymus vulgaris* L. that were extracted at different stages of plant growth.

MATERIALS AND METHODS

The thyme essential oils were obtained by distillation in a flow of steam from plants cultivated in the Comunalie Parmensi Consortium experimental fields at Borgotaro (Parma, Italy). In particular, the extracted oils were obtained from leaves of 3-year-old plants at the beginning of flowering (thyme starting to flower, TAF), 3-year-old plants in full flower (thyme in flower, TF), plants transplanted at the end of June and still not in flower (thyme in October, TO), and plants transplanted at the end of June but cultivated in a different place (thyme in Verona, TV).

One kilogram of plant materials, cut into small pieces, was placed in a distillation apparatus with 1.3 liters of double-distilled water and hydrodistilled for 1 h. After steam distillation, the oil was collected and stored at 4°C until used.

The essential oils under examination were tested on nine strains of gram-negative bacteria (*Escherichia coli* EC, *E. coli* O157:H7 VTEC, *Proteus mirabilis* PM, *P. vulgaris* PV, *Salmonella* Typhimurium STM, *Serratia marcescens* SM, *Yersinia enterocolitica* YE, *Pseudomonas fluorescens* PF, *P. putida* PP) and six strains of gram-positive bacteria (*Micrococcus* sp. M, *Sarcina flava* SF, *Staphylococcus aureus* SA, *B. licheniformis* BL, *Bacillus thuringiensis* BT, *Listeria innocua* LI), obtained from a collection at the Istituto di Ispezione degli Alimenti di Origine Animale at the University of Milan, Italy, and preserved in brain heart infusion agar (Oxoid, Italy) at 4°C.

The bioimpedance method was chosen for studying the antibacterial activity of the thyme essential oils; this records the variation of an electrical signal directly in the culture medium and

TABLE 2. *Detection times in the presence of various concentrations of* Thymus vulgaris *TF essential oils*[a]

	Control	50 ppm	100 ppm	200 ppm	400 ppm
Escherichia coli	3.4	5.0 F	6.8 G	18.8 EF	22.2 G
E. coli O157:H7	4.3	16.1 A	33.4 A	61.0 A	>100 A
Proteus mirabilis	2.8	3.7 GH	6.7 G	8.5 I	14.7 H
P. vulgaris	2.7	3.3 H	4.6 H	9.0 HI	16.2 H
Salmonella Typhimurium	3.0	4.5 FG	7.7 G	8.8 I	15.4 H
Serratia marcescens	3.0	3.1 H	4.7 H	5.3 L	8.6 I
Yersinia enterocolitica	6.0	15.6 A	22.3 B	30.2 B	71.4 B
Pseudomonas fluorescens	10.3	12.0 B	15.1 D	17.2 F	23.2 G
P. putida	8.5	9.0 D	11.0 EF	12.6 G	20.5 G
Micrococcus spp.	5.4	5.9 E	10.0 F	19.6 E	37.3 E
Sarcina flava	3.8	9.3 BD	17.8 C	27.1 C	71.1 B
Staphylococcus aureus	3.1	3.2 H	4.2 H	11.1 GH	27.2 F
Bacillus licheniformis	6.7	9.5 CD	18.0 C	26.7 C	54.9 C
B. thuringiensis	3.5	5.0 F	12.3 E	22.8 D	47.1 D
Listeria innocua	5.1	10.2 C	16.5 CD	28.0 C	45.0 D

[a] Data in the column followed by different letters are significantly different at $P < 0.01$ according to Tukey's HSD test.

TABLE 3. *Detection times in the presence of various concentrations of* Thymus vulgaris *TO essential oils*[a]

	Control	50 ppm	100 ppm	200 ppm	400 ppm
Escherichia coli	3.4	4.0 F	4.4 GH	6.0 I	9.1 F
E. coli O157:H7	4.3	7.5 CD	14.8 A	22.3 A	42.6 A
Proteus mirabilis	2.8	4.4 F	5.0 F	6.4 H	7.6 H
P. vulgaris	2.7	3.1 H	3.1 I	8.6 G	13.0 D
Salmonella Typhimurium	3.0	5.2 E	6.6 E	9.3 FG	16.4 C
Serratia marcescens	3.0	4.0 F	4.2 H	4.7 M	8.9 F
Yersinia enterocolitica	6.0	6.5 D	15.0 A	17.9 B	21.7 B
Pseudomonas fluorescens	10.3	11.1 A	13.9 AB	15.1 CD	20.3 B
P. putida	8.5	10.3 A	12.1 ABC	13.5 D	20.5 B
Micrococcus spp.	5.4	6.9 CD	8.6 D	10.6 C	17.6 C
Sarcina flava	3.8	4.1 F	4.7 FG	5.5 L	11.1 E
Staphylococcus aureus	3.1	3.5 G	6.9 E	9.6 F	16.8 C
Bacillus licheniformis	6.7	7.3 CD	11.0 BC	21.9 A	39.1 A
B. thuringiensis	3.5	8.0 BC	10.4 C	16.4 BC	20.2 B
Listeria innocua	5.1	9.2 AB	14.6 A	18.6 AB	39.7 A

[a] Data in the column followed by different letters are significantly different at $P < 0.01$ according to Tukey's HSD test.

supplies a measure of the metabolic activity of the microbial species present. The bioimpedance system used for evaluating the antibacterial activity of the essential oils was a Bactometer Microbial Monitoring System M-128 (Vitek Systems, bioMérieux, Marcy l'Etoile, France), a system that allows the simultaneous recording of the electrical signals from 128 wells, inside which the culture medium and the microorganisms are placed. In this experiment, the culture medium was GPM Plus (bioMérieux). The parameter chosen for defining and quantifying the antibacterial activity of the essential oils was the detection time (DT), defined as the time required by the microbial population to reach the concentration, defined as the threshold quantity, required to cause a rapid deviation in the curve of the percentage electrical variation; for given experimental conditions, DT is a function of the initial microbial concentration, the microbial generation time, and the length of the lag phase. In our case, the difficulty experienced by the microorganisms growing in the presence of the essential oils in comparison with the same populations growing in media without inhibitors (controls) is expressed as a delay in attaining the DT.

Prior to the inhibition experiments, the strains were allowed to grow in brain heart infusion broth (Oxoid) at 30°C for 24 h; suitable decimal dilutions (in sterile NaCl solution, 9 g liter^{-1}) of the microorganisms were then prepared. One milliliter of sterile GPM Plus and 0.1 ml of a suspension of the test microorganism was placed in each Bactometer well, in order to obtain an initial microbial concentration of 10^2 CFU/ml. The culture broths contained in the wells of the modules in the Bactometer then received a known quantity of essential oil (in ethanol) or ethanol (control) and were allowed to incubate in the thermostated spaces in the Bactometer system; the concentrations of essential oils were 50, 100, 200, and 400 ppm. The results were expressed as DT, and each value was the mean of at least triplicate measurements.

The temperatures were chosen to give the microorganisms optimum growth conditions (35°C for *Enterobacteriaceae, Bacillaceae, Micrococcaceae,* and *L. innocua* and 25°C for *Pseudomonadaceae*).

To evaluate the properties of some of the concentrations of essential oils in more detail, the effect of essential oils by direct contact was estimated; after treatment the microorganisms were exposed to an extremely rich medium to investigate whether the cells could recover and thus repair the damage suffered.

TABLE 4. *Detection times in the presence of various concentrations of* Thymus vulgaris *TV essential oils*[a]

	Control	50 ppm	100 ppm	200 ppm	400 ppm
Escherichia coli	3.4	4.0 G	4.3 G	4.5 G	8.7 I
E. coli O157:H7	4.3	5.1 F	11.9 CD	14.4 C	30.5 AB
Proteus mirabilis	2.8	3.0 H	3.5 H	3.5 G	8.5 I
P. vulgaris	2.7	4.7 F	5.5 F	6.1 F	15.5 G
Salmonella Typhimurium	3.0	4.8 F	5.6 F	5.9 F	18.5 F
Serratia marcescens	3.0	3.2 H	3.4 H	3.4 G	5.1 L
Yersinia enterocolitica	6.0	14.2 A	18.4 A	23.1 A	25.6 BC
Pseudomonas fluorescens	10.3	11.9 AB	13.3 BC	14.7 C	19.4 EF
P. putida	8.5	10.0 BC	10.4 DE	12.3 D	17.6 F
Micrococcus spp.	5.4	7.7 DE	10.2 DE	11.5 DE	26.9 ABC
Sarcina flava	3.8	8.0 CDE	15.8 AB	19.0 B	24.4 CD
Staphylococcus aureus	3.1	8.4 CD	9.7 E	10.6 E	12.7 H
Bacillus licheniformis	6.7	8.5 CD	11.1 CDE	12.4 D	21.6 DE
B. thuringiensis	3.5	11.5 AB	15.3 AB	18.3 B	32.9 A
Listeria innocua	5.1	6.9 E	10.4 DE	14.7 C	24.9 CD

[a] Data in the column followed by different letters are significantly different at $P < 0.01$ according to Tukey's HSD test.

TABLE 5. *Media and standard deviation of DT values of* Thymus vulgaris *essential oils at various concentrations*[a]

	50 ppm		100 ppm		200 ppm		400 ppm	
	Media	SD	Media	SD	Media	SD	Media	SD
TAF	7.3	3.7	10.4 AB	5.5	13.6 B	8.0	23.1 B	12.9
TF	7.7	4.3	12.7 A	7.9	20.4 A	13.6	39.0 A	27.5
TO	6.4	2.5	9.0 B	4.3	12.4 B	5.8	20.3 B	11.1
TV	7.4	3.3	9.9 AB	4.6	11.6 B	5.9	19.5 B	8.1

[a] Data in the column followed by different letters are significantly different at $P < 0.01$ according to Tukey's HSD test.

This experiment involved the preparation of suspensions of 10^5 CFU/ml bacterial cells in peptone water; 800, 400, and 200 ppm of essential oil in ethanol were then added to these suspensions. Inoculated suspensions without the essential oils, but containing appropriate concentrations of ethanol, served as controls. The suspensions were exposed to the oil in ethanol or to ethanol for 60 min, and then 1 ml of 10^{-2} dilution (in a sterile NaCl solution, 9 g liter^{-1}) was placed on duplicate petri plates and about 15 ml brain heart infusion agar at 45°C was added; the plates were then incubated for 24 to 48 h at the ideal temperature for the various microorganisms. The counts obtained were compared with those performed on control plates. At the same time, in order to establish the capacity of the microorganisms to recover, 1 ml of suspension was placed in petri dishes and about 15 ml brain heart infusion agar containing 50% wt/vol egg yolk emulsion (Oxoid) was added. The counts obtained were again compared with control plates. To determine percent inactivation and recovery, three replicates were performed.

The data obtained were statistically analyzed by the analysis of variance and the means separated according to Tukey's HSD test with a significance level of 0.01.

Gas-chromatographic analysis of the thyme essential oils was performed with a Carlo Erba 8560 series Mega2 gas chromatograph, fitted with a flame ionization detector and an SSL 71 split/splitless injector, connected to a Carlo Erba DP 700 integrator; the column used was a 60-m capillary column with an internal diameter of 0.32 mm and a film thickness of 0.25 μm. The chromatographic conditions were as follows: (i) split ratio 1:50; (ii) helium flow rate 1.70 ml/min; (iii) temperature program: 5 min at 50°C, ramp from 50°C to 140°C with a gradient of 4°C/min, second ramp from 140°C to 220°C with a gradient of 10°C/min, isothermal at 220°C; (iv) injector temperature 260°C; (v) detector temperature 260°C; (vi) sample preparation: 10 mg in 0.5 ml dichloromethane (Fluka); and (vii) injection volume: 0.3 μl.

The chemical analysis of the essential oils was rendered difficult by the complexity of the mixtures and the difficulty of obtaining adequate standards, so it was not considered appropriate to proceed with a quantitative analysis as this did not appear to be necessary for the aims of the work. It was considered sufficient simply to express the quantities of the various components in percentage areas: the area of a single component of an essential oil was evaluated as a ratio of the total area of the chromatogram of the oil in question. The identification of the peaks was possible by reference to the library of a Varian Saturn ion trap mass spectrometer (ion-trap detector), coupled to the gas chromatograph, and suitable references from the literature *(19, 25).*

RESULTS AND DISCUSSION

Tables 1 through 4 report the DT values obtained for the various microorganisms at the different concentrations of essential oils. Data in the column followed by different letters are significantly at $P < 0.01$ according to Tukey's HSD test.

As can be seen, the presence of the essential oils at all the concentrations tested produced an increase in the DT and a reduction in the rate of growth of the different microorganisms. The activity of the essential oils appeared to depend on the type and concentration of the oil, the collection period of the herb, and the type of microorganism tested.

All the thyme oils tested had good antibacterial activity, and this activity was more marked against the gram-positive bacteria. In fact, the DTs recorded in the presence of 400 ppm of essential oil were almost always more than 20 h for *S. flava, B. thuringiensis, B. licheniformis,* and *L. innocua,* while of the *Enterobacteriaceae* such high values were only registered for *Y. enterocolitica* and *E. coli* O157:H7 (Tables 1–4) and, in the case of the thyme in flower, for *E. coli,* for which it was not possible to record the DT within the first 100 h of incubation (Table 2).

Of the gram-negative bacteria, the two species of *Pseudomonadaceae* were noteworthy for their sensitivity to the thyme essential oils, with *P. fluorescens* being slightly more sensitive than *P. putida* in the case of *T. vulgaris* TAF at 400 ppm ($P < 0.01$) (Table 1). Of the gram positives, *S. aureus* appeared to be the most resistant of the species tested, together with *Micrococcus* spp. in the case of *T. vulgaris* TO at 400 ppm (Table 3).

The inhibitory effect due to the various thyme oils was good, given that the delay in recording the DT was on average about 5 h with respect to the controls, even at concentrations of 100 ppm thyme oil. As can be seen in Table 5, at a concentration of 50 ppm the effects of the essential oils were similar, while at 100 ppm the TF was the most efficient and TO the less active, TAF and TV having intermediate characteristics. At 200 and 400 ppm TF was the most active essential oil and TAF, TO, and TV the less efficient ($P < 0.01$).

E. coli O157:H7 was the most sensitive of the microbial species examined ($P < 0.01$); in the presence of 400 ppm of oil from TF, it was not possible to record a DT within the first 100 h of incubation (Table 2), indicating that this concentration probably had a bactericidal effect on *E. coli* VTEC. The most resistent strain was *S. marcescens* (Tables 1 through 4).

Table 6 reports the percentage inactivation recorded for each strain tested in the 60-min contact trials with the various concentrations of thyme essential oil.

TABLE 6. *Percentage of inactivation of microorganisms after contact with various concentrations of* Thymus vulgaris *TV essential oils*[a]

	TAF		TF		TO		TV	
	200 ppm	400 ppm	800 ppm	200 ppm	400 ppm	800 ppm	200 ppm	400 ppm	800 ppm	200 ppm	400 ppm	800 ppm
Escherichia coli	72 A	100 A	100 A	74 AB	100 A	100	66 B	90 A	100 A	63 A	91 A	100 A
E. coli O157:H7	98 A	100 A	100 A	96 A	100 A	100	97 A	97 A	100 A	86 A	97 A	99 A
Proteus mirabilis	47 ABC	85 BC	87 CD	51 BCD	99 AB	100	32 CD	81 A	88 D	21 CDE	76 A	69 E
P. vulgaris	47 ABC	90 AB	95 B	51 BCD	92 CD	100	42 C	83 A	1 C	12 EF	86 A	73 CDE
Salmonella Typhimurium	36 ABC	84 BC	93 B	37 DE	90 D	100	27 D	91 A	99 A	16 DEF	71 A	82 B
Serratia marcescens	21 C	60 E	82 D	40 CDE	65 G	100	13 E	38 D	82 E	10 F	38 C	73 CDE
Yersinia enterocolitica	35 ABC	61 E	96 AB	25 E	78 F	100	24 E	32 E	98 AB	21 CDE	34 C	80 BCD
Pseudomonas fluorescens	52 ABC	92 AB	87 C	75 AB	95 BC	100	43 E	82 A	87 D	29 BC	82 A	72 CDE
P. putida	64 AB	97 A	85 CD	74 AB	100 A	100	45 C	78 A	91 C	39 B	87 A	72 E
Micrococcus spp.	22 BC	85 BC	100 A	65 ABC	100 A	100	22 E	76 AB	99 AB	20 CDE	33 C	97 A
Sarcina flava	30 ABC	73 D	85 CD	52 BCD	93 CD	100	23 E	48 C	82 E	26 BCD	47 B	78 BCD
Staphylococcus aureus	22 BC	61 E	93 B	40 CDE	85 E	100	15 E	57 BC	96 B	14 DEF	38 C	71 DE
Bacillus licheniformis	8 D	97 A	100 A	12 F	99 A	100	3 F	38 D	100 A	2 G	33 C	77 BCD
B. thuringiensis	48 ABC	79 CD	100 A	41 CD	80 F	100	43 C	68 AB	100 A	66 A	72 A	99 A
Listeria innocua	75 A	93 AB	97 AB	85 A	100 A	100	76 B	88 A	100 A	75 A	84 A	96 A

[a] Data in the column followed by different letters are significantly different at $P < 0.01$ according to Tukey's HSD test.

The percent inactivation after treatment with the TF at a concentration of 800 ppm was always 100% against both gram-positive and gram-negative bacteria; the same concentration of the other three oils caused 100% inactivation of the cells only for certain species: in particular, *E. coli*, *E. coli* O157:H7, *B. licheniformis*, and *B. thuringiensis* were completely inactivated by the essential oils from thyme just coming into flower (TAF) and the October thyme (TO), while *L. innocua* was also very sensitive to contact with these two oils (Table 6).

TV was the least active essential oil by contact ($P < 0.01$), as only the two strains of *E. coli*, *B. thuringiensis*, and *L. innocua* were inactivated above 90%; in contrast, the action of TO at concentrations of 800 ppm was fairly strong against all the species tested, with percentage inactivation never lower than 80%.

At concentrations of 200 ppm of essential oil, *B. licheniformis* was the least sensitive and *E. coli* O157:H7 was again the most sensitive species, with a percentage inactivation on contact with all the oils tested never being lower than 86%. *L. innocua* also demonstrated a high degree of sensitivity to 200 ppm of essential oil, with percentage higher than 75%. A rather marked sensitivity to the lowest concentration of essential oil was also demonstrated by *E. coli* (inactivation higher than 60%) and *B. thuringiensis* (Table 6).

At concentrations of 200 and 400 ppm the essential oils of *T. vulgaris* TF and TAF were the most efficient in inactivating microorganisms, while at 800 ppm the efficiency of TF was higher than TAF ($P < 0.01$).

The test that checked the inactivation of the microbial species following contact with the essential oil from TF (the oil shown to be most efficient in the previous trials) at concentrations of 800 and 400 ppm was followed by a test aimed at assessing the possible recovery of metabolic activity after incubation in a medium containing an emulsion of egg; the results of this experiment are reported in Table 7.

As can be seen, the percentage recoveries after contact with TF at 800 ppm were lower than 35% for 11 of the 15 species tested; the most resistant species was *B. thuringiensis*, while complete inactivation (0% recovery) was suffered by *E. coli* O157:H7, *P. vulgaris*, *Y. enterocolitica*, *S. flava*, and *L. innocua*, species that are thus irreversibly damaged by contact with the essential oil.

At concentrations of 400 ppm of TF essential oil, the percentage recovery of the activity increased for all the species examined with the exception of *E. coli* O157:H7, which, even at this oil concentration, had no capacity to recover metabolic functions. *Y. enterocolitica* and *P. vulgaris* also had low recovery capacities after contact with 400 ppm of TF, which even at this concentration had good antibacterial activity against several of the microbial species tested (Table 7).

Table 8 presents the qualitative and percent composition of the thyme essential oils. The thyme oils had high percentages of phenolic compounds (thymol, carvacrol) that are responsible for the marked antimicrobial activity. In fact, the mode of action of phenolic compounds is generally thought to involve interference with functions of the

TABLE 7. *Percentage of inactivation and recovery after contact with* Thymus vulgaris *TF*

	400 ppm		800 ppm	
	% inactivation	% recovery	% inactivation	% recovery
Escherichia coli	100 A	48 C	100	3 E
E. coli O157:H7	100 A	0 F	100	0 E
Proteus mirabilis	99 AB	22 D	100	2 E
P. vulgaris	92 CD	11 E	100	0 E
Salmonella Typhimurium	90 D	72 AB	100	58 C
Serratia marcescens	65 G	69 AB	100	59 BC
Yersinia enterocolitica	78 F	12 E	100	0 E
Pseudomonas fluorescens	95 BC	61 ABC	100	33 D
P. putida	100 A	61 ABC	100	29 D
Micrococcus spp.	100 A	44 C	100	6 E
Sarcina flava	93 CD	46 C	100	0 E
Staphylococcus aureus	85 E	78 A	100	28 D
Bacillus licheniformis	99 A	84 A	100	65 B
B. thuringiensis	80 F	76 A	100	75 A
Listeria innocua	100 A	51 BC	100	0 E

[a] Data in the column followed by different letters are significantly different at $P < 0.01$ according to Tukey's HSD test.

cytoplasmatic membrane, including proton motive forces and active transport *(11, 16).*

The compound present in the highest percent in the essential oils of thyme was thymol (averaging 31.19%), followed by γ-terpinene (24.73%) and *p*-cymene (15.79%). The percentage of these compounds varied slightly in the different collection periods, but the sum of the relative percentages of the compounds remained almost constant, as reported by Senatore *(28).*

Chemical analysis data were unable to explain the greater antimicrobial efficiency of the essential oil in TF; however, it is probable that this property is due to some minor component that may be involved in some type of synergism with the other active compounds.

CONCLUSIONS

The bioimpedance technique, adopted to test the antibacterial activity of the essential oils, was found to be very useful for identifying both the presence of bactericidal and bacteriostatic activity in essential oils of thyme against the microbial species considered. In addition to providing complete results on the activities of these oils, the application of this technique allowed a great deal of work to be performed in a very short period while also providing reliable results.

All the thyme essential oils examined were demonstrated to have a good degree of bacteriostatic activity against the microorganisms tested in this study. This activity was more marked against the gram-positive bacteria; of the gram-negative bacteria, the most sensitive were *E. coli* O157:H7 and *Y. enterocolitica,* while *L. innocua* was the most sensitive gram-positive bacterium. The oil from thyme in full flower (TF) was without exception the most active at slowing the growth of the microbial species examined (Table 5), followed by thyme just coming into flower, thyme grown near Verona, and thyme harvested in October.

TABLE 8. *Qualitative and percent composition (expressed as %) of* Thymus vulgaris *L.*

	TAF	TF	TO	TV	Media (%)	Coefficent of variation (%)
α-Pinene	1.12	1.19	0.83	1.14	1.07	13.2
α-Thujone	2.00	2.07	1.45	1.58	1.77	14.9
Camphene	0.59	0.68	0.64	0.73	0.63	11.7
1-Octen-3-ol	0.75	0.79	1.12	1.17	0.96	19.7
Myrcene	3.31	3.27	2.35	2.71	2.91	13.8
α-terpinene	3.16	2.49	1.95	2.30	2.47	17.8
p-Cymene	10.64	18.81	14.89	18.84	15.79	21.4
1,8-Cineol	1.16	1.43	1.05	1.13	1.19	12.0
γ-Terpinene	30.95	22.60	21.79	23.60	24.73	14.7
Cis-*p*-menten-1-ol	0.75	0.64	1.11	0.59	0.77	26.3
Camphor	0.33	0.32	0.54	0.70	0.47	33.4
Linalool	2.57	2.68	2.30	2.38	2.48	6.0
Borneol	0.68	0.54	0.63	0.64	0.62	8.2
Terpinen-4-ol	1.26	1.71	1.48	1.73	1.54	12.4
Thymol	33.74	33.40	41.06	32.55	31.19	9.7
Carvacrol	1.73	1.58	1.89	2.62	1.95	20.4

The oils tested were also shown to have good antibacterial activity by direct contact, which appeared to be more marked against the gram-negative bacteria; of the gram-positive bacteria, the most sensitive species, even to the lowest oil concentrations, were *L. innocua* and the *Bacillaceae.*

The experiment that checked the possibility of a recovery of metabolic activity after contact with the inhibitor demonstrated that only a few of the species were capable of recovering at least 50% of their metabolic function, while most members of the strains were shown to have been inactivated almost completely. *E. coli* O157:H7 was the most sensitive species, given that after contact with even the lowest concentration of oil it had a percentage recovery of 0%.

The results obtained in this experiment allow us to hypothesize the mode of action of the essential oils against the individual microbial species tested that could be capable of recovering after contact with a rich medium only when the damage caused by the essential oil was limited to damage to proteins in the membrane, without interference with the phospholipid components that would lead to irreversible damage to the integrity of the membranes.

Essential oil of *T. vulgaris* L. is classified as generally recognized as safe and is permitted in food; however, because of its characteristic flavor, the use of essential oils of *T. vulgaris* as food preservatives may be restricted to flavored foods and to those containing spices.

A reduction in the dosage of the essential oil in food systems may be attained by making use of the added inhibitory effect of low temperature storage *(27).*

REFERENCES

1. Aktug, S. E., and M. Karapinar. 1986. Sensitivity of some common food-poisoning bacteria to thyme, mint and bay leaves. Int. J. Food Microbiol. 3:349–354.
2. Aureli, P., A. Costantini, and S. Zolea. 1992. Antimicrobial activity of some plant essential oils against *Listeria monocytogenes.* J. Food Prot. 55:344–348.
3. Bahk, J., A. E. Yousef, and E. H. Marth. 1990. Behaviour of *Listeria monocytogenes* in the presence of selected spices. Lebensm. Wiss. Technol. 23:66–69.
4. Beuchat, L. R. 1976. Sensitivity of *Vibrio parahaemolyticus* to spices and organic acids. J. Food Sci. 41:899–902.
5. Beuchat, L. R., and D. A. Golden. 1989. Antimicrobials occurring naturally in foods. Food Technol. 43:134–142.
6. Canillac, N., and A. Mourey. 1996. Comportement de *Listeria* en présence d'huiles essentielles de sapin et de pin. Sci. Aliment. 16: 403–412.
7. Chaibi, A., L. H. Ababouch, K. Belasri, S. Boucetta, and F. F. Busta. 1997. Inhibition of germination and vegetative growth of *Bacillus cereus* T and *Clostridium botulinum* 62A spores by essential oils. Food Microbiol. 14:161–174.
8. Conner, D. E., and L. R. Beuchat. 1984. Effects of essential oils from plants on growth of food spoilage yeasts. J. Food Sci. 49:429–434.
9. Daouk, R. K., S. M. Dagher, and E. J. Sattout. 1995. Antifungal activity of the essential oil of *Origanum syriacum* L. J. Food Prot. 58:1147–1149.
10. Deans, S. G., and K. P. Svodoba. 1989. Antibacterial activity of summer savory (*Satureja hortensis* L.) essential oil and its constituents. J. Hortic. Sci. 64:205–210.
11. Eklund, T. 1985. The effect of sorbic acid and esters of p-hydroxybenzoic acid on the protonmotive force in *Escherichia coli* membrane vesicles. J. Gen. Microbiol. 131:73–76.
12. El-Khateib, T., and H. Abd El-Rahman. 1987. Effect of garlic and *Lactobacillus plantarum* on growth of *Salmonella typhimurium* in Egyptian fresh sausage and beefburger. J. Food Prot. 50:310–311.
13. Farag, R. S., Z. Y. Daw, F. M. Hewedi, and G. S. A. El-Baroty. 1989. Antimicrobial activity of some Egyptian spice essential oils. J. Food Prot. 52:665–667.
14. Farag, R. S., H. Salem, A. Z. M. A. Badei, and D. E. Hassanein. 1986. Biochemical studies on the essential oils of some medicinal plants. Fette Seifen Anstrichm. 88:69–72.
15. Frazier, W. C., and D. C. Westhoff. 1988. Food microbiology, 4th ed. McGraw Hill, New York.
16. Freese, E., C. W. Shen, and E. Galliers. 1973. Function of lipophilic acids as antimicrobial food additives. Nature 241:321–327.
17. Hefnawy, Y. A., S. I. Moustafa, and E. H. Marth. 1993. Sensitivity of *Listeria monocytogenes* to selected spices. J. Food Prot. 56:876–878.
18. Holt, D. L., and N. Gomez Almonte. 1995. Anti-mycotic activity of garlic extracts and extract fractions in vitro and in planta. J. Food Prot. 58:322–325.
19. Jennings, W., and T. Shibamoto. 1980. Qualitative analysis of flavor and fragrances volatiles by glass-capillary gas-chromatography. Academic Press, New York.
20. Karapinar, M. 1990. Inhibitory effects of anethole and eugenol on the growth and toxin production of *Aspergillus parasiticus.* Int. J. Food Microbiol. 10:193–200.
21. Karapinar, M., and S. E. Aktug. 1987. Inhibition of foodborne pathogens by thymol, eugenol, menthol and anethole. Int. J. Food Microbiol. 4:161–166.
22. Kubota, K., S. Ohhira, and A. Kobayashi. 1994. Identification and antimicrobial activity of the volatile flavor constituents from *Scorodocarpus borneensis* Becc. Biosci. Biotechnol. Biochem. 58:644–646.
23. Larpent, J. P. 1995. Les Listeria. Lavoisier Tec. et Doc., Paris.
24. Libby, J. A. 1975. Meat hygiene, 4th ed. Lea and Febiger, Philadelphia.
25. McGimpsey, J. A., M. H. Douglas, J. W. van Klink, B. Perry, and N. B. Perry. 1994. Seasonal variation in essential oil yield and composition from naturalized *Thymus vulgaris* L. in New Zealand. Flavour Fragrance J. 9:347–352.
26. Pandit, V. A., and L. A. Shelef. 1994. Sensitivity of *Listeria monocytogenes* to rosemary (*Rosmarinus officinalis* L.). Food Microbiol. 11:57–63.
27. Scott, V. N. 1989. Interaction of factors to control microbial spoilage of refrigerated foods. J. Food Prot. 52:431–435.
28. Senatore, F. 1996. Influence of harvesting time on yield and composition of the essential oil of a thyme (*Thymus pulegioides* L.) growing wild in Campania (Southern Italy). J. Agric. Food Chem. 44:1327–1332.
29. Sivropoulou, A., E. Papanikolaou, C. Nikolaou, S. Kokkini, T. Lanaras, and M. Arsenakis. 1996. Antimicrobial and cytotoxic activities of *Origanum* essential oils. J. Agric. Food Chem. 44:1202–1205.
30. Ting, W. T. E., and K. E. Deibel. 1992. Sensitivity of *Listeria monocytogenes* to spices at two temperatures. J. Food Saf. 12:129–137.
31. Walton, L., M. Herbold, and S. Lindgren. 1936. Bactericidal effects of vapors of crushed garlic. Food Res. 1:163–169.
32. Yoshida, S., S. Kasuga, N. Hayashi, T. Ushiroguchi, H. Matsuura, and S. Nakagawa. 1987. Antifungal activity of ajoene derived from garlic. Appl. Environ. Microbiol. 53:615–617.
33. Zaika, L. L., and J. C. Kissinger. 1981. Inhibitory and stimulatory effects of oregano on *Lactobacillus plantarum* and *Pediococcus cerevisiae.* J. Food Sci. 46:1205–1210.
34. Zaika, L. L., T. E. Zell, S. A. Palumbo, and J. L. Smith. 1978. Effect of spices and salt on fermentation of Lebanon bologna-type sausages. J. Food Sci. 43:186–189.