Mycobiology 34(4): 219-229 (2006)

Antimicrobial Activity of Some Essential Oils Against Microorganisms Deteriorating Fruit Juices

G. A. Helal[1*], M. M. Sarhan[1], A. N. K. Abu Shahla[2] and E. K. Abou El-Khair[2]

[1]*Botany Department, Faculty of Science, Zagazig University, Sharkia Governorate, Egypt*
[2]*Biology Department, Faculty of Science, Al Azhar University- Gaza, Gaza strip, Palestine*

(*Received September 14, 2006*)

Seventeen microbial species including 10 fungal taxa, two yeasts and five bacteria, were isolated from freshly prepared orange, guava and banana juices kept in open bottles at room temperature for 7 days. Eight different essential oils, from local herbs, were tested for their antimicrobial activity against these test organisms. The essential oils of *Cymbopogon citratus*, *Ocimum basilicum* and *Origanum majorana* were found to be highly effective against these microorganisms. *Aspergillus niger*, *A. flavus* and *Saccharomyces cerevisiae*, the most prevalent microorganisms in juice, showed the highest resistance against these essential oils. GC-MS analysis showed that while e-citral, a'-myrcene, and z-citral represent the major components (75.1%) of the essential oil of *Cymbopogon citratus*; bezynen,1-methyl-4-(2-propenyl), 1,8-cineole and trans-a'-bisabolene were the main components (90.6%) of *Ocimum basilicum*; whereas 3-cyclohexen-1-01,4-methyl-1(1-methylethyl)-(CAS), c-terpinene and trans-caryophyllene represent the major components (65.1%) of *Origanum majorana*. These three essential oils were introduced into juices by two techniques namely, fumigation and direct contact. The former technique showed more fungicidal effect than the latter one against *A. flavus*, *A. niger*, and *S. cerevisiae*. The essential oil of *Cymbopogon citratus* by comparison to other test oils showed the strongest effect against these fungi with a minimum inhibitory concentration of 1.5 *μl/ml* medium and a sublethal concentration of 1.0 *μl/ml*. The antimicrobial activity of this oil is thermostable at 121°C for 30 min.

KEYWORDS: Antimicrobial activity, Bacteria, Essential oils, Fungi, Yeast

Essential oils of plants and their main components show antimicrobial activity against a wide range of microorganisms including antibiotic-resistant species of bacteria and fungi (Alviano *et al.*, 2005; Carson and Riley, 1995; El-Kabouss *et al.*, 2002; El-Kady *et al.*, 1993). They can also affect both yeast and filamentous fungi (Bishop and Thornton, 1997; Delaquis *et al.*, 2002; Gowda *et al.*, 2004; Krauze-Baranowska *et al.*, 2002; Vagi *et al.*, 2005) in addition to Gram-positive and Gram-negative bacteria (Ali *et al.*, 2002; Sechi *et al.*, 2001). Variable results have been observed depending on origin of antimicrobial substance; testing conditions and target microorganisms. The essential oil of *C. citratus* completely inhibited the growth of *Neurospora sitophila*, *Penicillium digitatum*, and *Aspergillus parasiticus* (Shadab *et al.*, 1992), and also exhibited fungal toxicity against *Aspergillus flavus* (Mishra and Dubey, 1994). Its aqueous extracts completely inhibited the growth of *Macrophomina phaseolina* and *Botryodiplodia theobramae*, while it significantly reduced the growth of *Gibberella fujikuroi* and *Fusarium solani* (Bankole and Adebanjo, 1995). EL-Kamali *et al.* (1998) noticed that the essential oils of *Nigella sativa* seeds, *Cymbopogon citratus* leaves and *Pulicaria undulata* aerial parts (collected from Sudan) exhibited activity against *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli* and *Pseudomonas aeruginosa.* Recently Mejlholm and Dalgaard (2002) found that oils of Oregano and Cinnamon showed the strongest antimicrobial activity against *Photobacterium phosphorium*, followed by lemongrass, thyme, clove, bay, marjoram, sage and basil oils. The essential oil of *Origanum majorana* L., at the concentration of 1ml/ml, inhibited both *A. niger* and *Trichoderma viride* (Vagi *et al.*, 2005).

The present investigation aimed at studying the antimicrobial activity of essential oils from some Egyptian plants against molds, yeasts and bacteria associated with the contamination of fruit juices and accordingly the possibility of using these oils as natural preservatives.

Materials and Methods

Juices. They were freshly prepared from fresh fruits of orange, guava, and banana collected from the local market of Zagazig City, Sharkia Governorate, Egypt. Fruits were washed several times with water, dried and peeled with a sterile knife. Under clean conditions berries were squeezed using handle machines or electrical blenders. The resultant juice was filtered through pasteurized double layers of cheesecloth and stored for seven days at room temperature in opened presterilized juice bottles.

*Corresponding author <E-mail: dr_helal56@hotmail.com>

Source of essential oils: The essential oils of *Ocimum basilicum* L. (Basil), *Carium carvi* L. (Caraway), *Foeniculum vulgare* Mill. (Fennel), *Pelargonium radula* L. Herit (Geranium), *Cymbopogon citratus* Stapf (lemongrass), *Origanum majorana* L. (Origanum), *Mentha piperita* L. (Peppermint) and *Thymus capitatus* (L.) Hoffmg. & Link (Thyme), were kindly supplied by Sekem Company, Hikstep, Cairo Governorate, Egypt.

Isolation. Bacteria, yeast and molds were isolated from deteriorated fruit juices using the dilution plate method (Johnson *et al.*, 1959) on nutrient, Sabouraud's and Czapek's agar media respectively. Serial dilutions (from 10^{-2} to 10^{-5}) were prepared from each juice. One ml aliquots from the appropriate dilution were transferred aseptically to each sterile Petri-dish. Agar plates of the three mentioned media were poured aseptically into the Petri-dishes. Five replicates for each dilution from each media.

The agar plates were incubated at 37°C for 24 hr in case of bacteria and yeast and 28°C for 5 days in case of filamentous fungi. Developing colonies of bacteria, yeasts and molds were counted, identified, and pure colonies were obtained.

Culture media. Thee media namely, nutrient agar, Sabouraud's and Czapek's were prepared according to Gams *et al.* (1998). Nutrient medium (g/*l*); bactopeptone (Difco), 5.0; beef extract (Difco), 5.0 and NaCl, 5.0. Sabouraud's medium (g/*l*); peptone (Difco), 10.0; glucose, 40.0; KH_2PO_4, 1.0; $MgSO_4 \cdot 7H_2O$, 0.5. Czapek's medium (g/l); sucrose, 30.0; $NaNO_3$, 3.0; KH_2PO_4, 1.0; KCl, 0.5; $MgSO_4 \cdot 7H_2O$, 0.5. Agar 15 g/*l* was added for solidification. The media are sterilized by autoclaving at 121°C for 15 min, unless stated otherwise. The pH of the media was adjusted to 7.0 for bacteria, 6.5 yeast growth and 5.5 for fungal growth before autoclaving. To suppress bacterial growth in case of yeast and molds isolation, 1 *ml* of Streptomycin solution was added to each Petri-dish before pouring media, to give as final concentration of 30~35 ppm.

Identification of microbial isolates. *Bacillus subtilis*, *B. cereus*, *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas* sp. were identified according to Bergey's manual of determinative bacteriology (Holt *et al.*, 1986). *Saccharomyces cerevisiae* and *Candida albicans* were identified according to Barnett *et al.* (2000). Fungal isolates were purified using single spore technique and identified according to Domsch *et al.* (1980), Kitch and Pitt (1992), Moubasher (1993), Pitt (1979), Pitt (1986) Raper and Fennell (1977) and Raper and Thom (1968).

Antimicrobial assay of essential oils.

Preparation of inocula: Inocula were prepared by growing bacterial cells in nutrient broth medium and yeast cells in Sabouraud's medium at 37°C for 24 hr. These cell suspensions were diluted with the same broth medium to provide initial cell counts of about 10^5 CFU (colony forming unit)/*ml*. An aliquot of 1 *ml* is used each experiment. Test fungi were cultured on Czapek's medium, where each flask was inoculated with a mycelial disc of 5 mm diameter of five days old fungal culture.

Screening for the antimicrobial effect of essential oils: Well cut diffusion method was used in this survey. Culture plates seeded with the desired tested organisms were used in this test. Holes of "1 cm diameter" were cut using a sterilized cork borer. After which drops of water agar (15 g/*l*) were put in holes, then 50 μl of each essential oil were introduced into each hole. The plates were put in the refrigerator for 2 hr and incubated at 37°C for 24 hr for yeast and bacteria and at 28°C for 5 days for fungi. After incubation, plates were viewed and the diameters of inhibition zones were determined.

Determination of minimal inhibitory concentration (MIC) of essential oils: The MIC of each oil under test was determined using two techniques namely, contact and fumigation methods.

The contact method: Broth media were prepared and sterilized in 100 *ml* capacity conical flasks, each containing 20 *ml* of the culture medium. Different amounts of the essential oils under test were added to sterilized broth medium to give the following concentration per *ml*: 10, 5, 4, 3, 2, 1.5, 1 and 0.5 μl. One *ml* Tween-80/*l* broth medium was added as emulsifying agent. Tween-80 (1 *ml*/*l*) did not exhibit antifungal activity. Flasks were then inoculated with one disc of 5 mm diameter for each flask of seven day old fungal culture of test fungi. While in case of yeast, flasks were inoculated with 1 *ml* cell suspension to give about 10^5 CFU/*ml* from 48 hr old culture. Flasks were then incubated at 28°C for 5 days for fungal growth and at 37°C for 24 hr for yeast cultures.

Standard curve of turbidity and cell number (per *ml*) was made for yeast by growing them under the same culture condition (without addition of any of the essential oils). Number of cells per *ml* was counted using serial dilution method as previously mentioned. At the end of the incubation period, the optical density (O.D) for yeast cultures was determined at 660 nm (using Spectronic 20 Spectrophotometer) then the cell density was calculated referring the standard curve. On the other hand, fungal growth was filtered through preweighted Whatman No. 1 filter paper. The growth of fungi was estimated gravimetrically by weighting the biomass after having dried at 80°C until constant weights were reached. The MIC was determined from the lowest concentration at which no growth occurs.

The fumigation method: The details of this method were similar to these of the contact method except for the mode of application of the oil and the absence of Tween-80. Hence, the desired amount of the essential oil was aseptically absorbed on a piece of round, sterile filter paper suspended at the top of flasks.

Percentage of inhibition for filamentous fungi

$$\frac{\begin{array}{l}\textit{Net}\ \text{dry weight of control cells}\\ -\textit{Net}\ \text{dry weight of treated cells}\end{array}}{\textit{Net}\ \text{dry weight of control cells}} \times 1$$

Percentage of inhibition for yeast

$$\frac{\text{No of control cells} - \text{No of treated cells}}{\text{No of control cells}} \times 1$$

Determination of the main components of the most effective essential oils: The main components of three oils showing the greatest activity were determined by chemical analyses using Gas Chromatography/Mass Spectrometry (GC/MS) model Vinigan Mat SQQ 7000 at The National Research Center, Giza, Egypt.

Fungicidal-fungistatic nature of essential oils. Fungicidal-fungistatic nature of the oils was detected by the technique of Thompson (1989). According to which one ml of each of oil-inhibited yeast or mold broth medium was reinoculated onto plates of Sabouraud's or Czapek's agar medium and the revival of growth has been recorded. The appearance of new growth means this concentration of oil is fungistatic and the absence of growth means the concentration is fungicidal.

Investigation of the thermal stability of *C. citratus* essential oil: Discs of filter paper impregnated with sub-lethal concentrations SLC and MIC of *C. citratus* essential oil were suspended in the flasks containing media before and after autoclaving at 1.5 atmosphere for 30 min. At the end of the experiment the antifungal activity of the oil was determined as percentage of growth inhibition.

Results

Fungi and bacteria of deteriorated juice. A total of 12 genera and 17 species of microorganisms were isolated from deteriorated juices (Table 1). Orange showed a total colony count of (all organisms) 16.6×10^4, guava 7.9×10^4 and banana 6.68×10^4 CFU/*ml*.

Fungal counts/ml juice in descending order was 1×10^4 for guava, followed by 0.7×10^4 for banana, and 0.6×10^4 for orange. These counts belonged to 10 taxa. As for genera isolated, *Aspergillus* was the most predominant fungus by showing total counts of 3.6×10^3, 6.9×10^3 and 3.3×10^3 respectively from orange, guava and banana juices. It was represented by 4 species namely; *A. candidus*, *A. flavus*, *A. niger* and *A. oryzae*. Each of these species was isolated from the three juices under test. The second genus in view of total count was *Penicillium*. It showed total counts of 1.7×10^3, 0.9×10^3 and 0.7×10^3/*ml* in orange, guava and banana juices, respectively. It represented by only 2 species of which *P. digitatum* from orange and guava juice with total counts 1.3×10^3 and 0.9×10^3/*ml* and *P. puberulum* isolated from orange and banana juices with total counts of 4×10^2 and 7×10^2/*ml*, respectively. The remaining genera were represented by one species each. *B. cinerea* was isolated from guava and banana juices, *C. herbarum*, *Mucor* sp. and *P. lilacinus* were isolated from the three juices under investigation.

Table 1. Microbial counts and changes of pH of fresh juice exposed to air for seven days at room temperature

Isolates	Count/*ml* juice		
	Orange	Guava	Banana
Aspergillus candidus Link	$3 \times 10^2 \pm 26$	$12 \times 10^2 \pm 200$	$6 \times 10^2 \pm 39$
Aspergillus flavus Link	$12 \times 10^2 \pm 170$	$30 \times 10^2 \pm 180$	$14 \times 10^2 \pm 82$
Aspergillus niger Van Tieghem	$17 \times 10^2 \pm 115$	$20 \times 10^2 \pm 130$	$11 \times 10^2 \pm 95$
Aspergillus oryzae (Ahlb.) Cohn	$4.2 \times 10^2 \pm 95$	$7 \times 10^2 \pm 70$	$2 \times 10^2 \pm 41$
Botrytis cinerea Persoon	–	$4 \times 10^2 \pm 26$	$4 \times 10^2 \pm 75$
Cladosporium herbarum (Pers.) Link	$2 \times 10^2 \pm 38$	$3 \times 10^2 \pm 56$	$4 \times 10^2 \pm 33$
Mucor sp.	$3 \times 10^2 \pm 36$	$7 \times 10^2 \pm 67$	$8 \times 10^2 \pm 57$
Penicillium digitatum (Pers. ex Fr.) Sacc.	$13 \times 10^2 \pm 96$	$9 \times 10^2 \pm 60$	–
Penicillium puberulum Bainier	$4 \times 10^2 \pm 31$	–	$7 \times 10^2 \pm 77$
Paecilomyces lilacinus (Thom) Samson	$3 \times 10^2 \pm 35$	$8 \times 10^2 \pm 49$	$12 \times 10^2 \pm 75$
Total count of filamentous fungi	$0.6 \times 10^4 \pm 642$	$1.0 \times 10^4 \pm 838$	$0.7 \times 10^4 \pm 574$
Bacteria	$7.5 \times 10^4 \pm 2200$	$3.5 \times 10^4 \pm 1900$	$1.8 \times 10^4 \pm 980$
Yeast	$8.5 \times 10^4 \pm 4000$	$3.4 \times 10^4 \pm 2150$	$4.2 \times 10^4 \pm 1000$
Total count of microorganisms	$16.6 \times 10^4 \pm 6842$	$7.9 \times 10^4 \pm 4888$	$6.7 \times 10^4 \pm 2554$
pH (initial)	3.8	3.6	4.3
pH (final)	4.1	3.8	4.5

The values are mean of five replicates ± standard deviation.

Molds isolated on Czapek agar medium pH 5.5, yeasts isolated on Sabouraud's agar medium pH 6.5 and bacteria isolated on Nutrient agar medium pH 7.

Table 2. Antimicrobial activity as diameter of inhibition zone (mm) of the tested essential oils 50 μl/hole against molds, yeasts and bacteria isolated from fresh juices of orange, guava and banana

Organism	Diameter of inhibition zone/mm of essential oils extracted from							
	Cymbopogon citratus	*Ocimum basilicum*	*Origanum majorana*	*Thymus capitatus*	*Mentha piperita*	*Foeniculum vulgare*	*Pelargonium radula*	*Carium carvi*
A. candidus	65 ± 4	50 ± 6	31 ± 3	42 ± 3	36 ± 3	25 ± 3	25 ± 2	22 ± 2
A. flavus	50 ± 5	24 ± 2	29 ± 3	30 ± 3	29 ± 2	26 ± 3	22 ± 2	–
A. niger	46 ± 4	35 ± 4	27 ± 2	26 ± 2	31 ± 3	22 ± 3	15 ± 2	22 ± 2
A. oryzae	66 ± 3	24 ± 2	24 ± 5	28 ± 3	37 ± 4	25 ± 2	14 ± 2	23 ± 3
Botrytis cinerea	120	120	120	120	120	120	120	120
C. herbarum	65 ± 3	60 ± 5	50 ± 4	55 ± 4	48 ± 4	33 ± 3	28 ± 3	41 ± 3
Mucor sp.	55 ± 4	49 ± 5	34 ± 4	47 ± 3	44 ± 3	29 ± 3	28 ± 2	26 ± 3
P. digitatum	41 ± 4	32 ± 3	28 ± 3	36 ± 3	33 ± 3	15 ± 3	18 ± 3	18 ± 2
P. puberulum	37 ± 3	33 ± 2	27 ± 3	28 ± 4	34 ± 3	17 ± 2	20 ± 3	27 ± 3
Paecilomyces lilacinus	60 ± 4	44 ± 4	27 ± 2	43 ± 3	36 ± 3	–	28 ± 3	14 ± 2
C. albicans	25 ± 2	28 ± 2	23 ± 2	30 ± 3	23 ± 2	12 ± 2	14 ± 2	18 ± 2
S. cerevisiae	28 ± 3	31 ± 2	24 ± 2	36 ± 3	22 ± 2	12 ± 2	13 ± 2	20 ± 2
B. subtilis	45 ± 4	42 ± 3	30 ± 3	54 ± 3	30 ± 3	26 ± 2	18 ± 2	28 ± 3
B. cereus	66 ± 4	54 ± 4	51 ± 3	50 ± 3	48 ± 4	33 ± 3	36 ± 3	45 ± 3
S. aureus	42 ± 3	36 ± 3	30 ± 3	36 ± 4	30 ± 3	32 ± 3	26 ± 3	29 ± 2
E. coli	58 ± 4	40 ± 4	36 ± 3	39 ± 4	44 ± 3	30 ± 4	28 ± 3	38 ± 3
Pseudomonas sp.	48 ± 3	48 ± 3	37 ± 3	36 ± 4	32 ± 3	26 ± 3	28 ± 3	36 ± 3

The values are mean of three replicates ± standard deviation.

Yeasts with total counts of 8.5×10^4, 3.4×10^4 and 4.2×10^4/*ml* of orange, guava and banana juices, respectively were predominant and represented by *C. albicans* and *S. cerevisiae.*

Total counts of 7.5×10^4, 3.4×10^4 and 1.8×10^4/*ml* bacteria were isolated from orange, guava and banana juices and were represented with 4 genera (two Gram-positive and two Gram-negative). The two Gram-positive genera namely; *Bacillus*, represented by *B. subtilis* & *B. cereus* and *S. aureus*, while the two Gram-negative genera were *E. coli* and *Pseudomonas* sp.

Antimicrobial activity of essential oils against fungi and bacteria isolated from fruit juices: Data presented in Table 2 indicated that all essential oils under test process showed antimicrobial activity against molds, yeasts and bacteria. Most of these oils delayed conidiation of fungi. The feature of antimicrobial activity varied not only from one essential oil to another but also among microorganisms. The antimicrobial activity of all tested oils was generally higher against bacteria than fungi and yeast. *Botrytis cinerea* and *Bacillus cereus* revealed the highest sensitivity to essential oils, while *P. digitatum* and *Candida albicans* revealed the lowest sensitivity. *B. cinerea* failed completely to grow in the presence of any of the eight essential oils. The oil of lemongrass (*Cymbopogon citratus*) was the most active against both molds and bacteria. In view of antimicrobial activity the oil of basil (*Ocimum basilicum*) comes next to lemongrass followed by thyme (*Thymus capitatus*) and origanum (*Origanum majorana*). By comparison, the remaining four essential oils showed weaker effects. In descending order activity, they came as follows: *Mentha piperita*, *Foeniculum vulgare*, *Pelargonium radula*, and *Carium carvi*.

The data in the present part of study revealed that the tolerance to the essential oils under test was found to be higher in filamentous fungi than that of bacteria and yeast fungi. Based on this observation the present study was extended to investigate the effect of these oils on the growth of *A. flavus*, *A. niger* and *S. cerevisiae* as test organisms (for being very common).

Analysis of the most effective oils: Given in Table 3~5 the main components of the essential oils of the three plants; *Cymbopogon citratus*, *Ocimum basilicum* and *Origanum majorana*. Where the data clearly indicated that the components are much different and the number of components in common is very low.

Table 3 showed that lemongrass contained 19 compounds accounting for 94.8% of the total oil components. E-citral is the major component of this oil (65.4%). Table 4 revealed that basil oil comprised 17 compounds constituting 98.19% of the total oil components. Bezynen,1-methyl-4-(2-propenyl) is the major component (80.1%). Table 5 showed that the oil of origanum contain a range of also 17 compounds accounting for 78.97% of total oil components. 3-cyclohexen-1-01,4-methyl-1(1-methylethyl)-(CAS) is the major component (51.5%).

Minimum inhibitory concentration (MIC): In Table 6 the inhibition effect of essential oils on the test fungi was studied. Oils were introduced in different concentrations as fumigants and as contact materials. The data of this Table clearly indicated that fumigation as a technique is more effective than using the contact method. Growth of

Table 3. Percentages of components of essential oil extracted from *Cymbopogon citratus*

Component	Percentage
e-citral	65.4
a'-Myrcene	6.7
z-citral	3.0
1,2 cis-1,5-trans-2,5-dihydroxy-4-methyl-1-(1-hydroxy-1-isopropyl) cyclohex-3-ene	2.9
2-tridecanone (CAS)	1.9
(−)-Caryophyllene oxide	1.6
Geranyl acetate	1.6
8-Bromoneois olongifolene	1.4
Nerolidol	1.4
2-Methyl-4,5,6,7-tetrahydroisoidol: N-1-One	1.2
Junipercamphor	1.2
Oxiranecarboxaldehyde-3-methyl-3-(4-methyl-3-pentenyl)-(CAS)	1.1
Neric acid	1.0
Linalool	0.9
3,7-Nouadiene-2-01,4,8-dimethyl	0.8
1H-Benzocyclohepten-7,01,2,3,4,4a,5,6,7,8-actahydro-1,1,4a,7-tetramethyl-,cis (CAS)	0.8
4,8,13-cyclotetradecatriene-1,3-diol,1,5,9-trimethyl-12-(1-methylethyl)-(CAS)	0.7
1-Cyano-4,9-dihydro-3-methyl-2-phenylpyrrolo [1,2-6] isoquinoline	0.5
Farnesol	0.4
2,5-Farandione, 3 (dodecenyl) dihydro	0.3
Total	94.8%

Table 4. Percentages of components of essential oil extracted from *Ocimum basilicum*

Component	Percentage
Bezynen,1-methyl-4-(2-propenyl)	80.1
1,8-cineole	6.7
Trans-a'-Bisabolene	3.8
Para methyl cinnamic aldehyde	2
(−)-caryophyllene oxide	1.6
Ë-cadinene	1.45
2-propenal,3-(3,4-dimethoxyphenyl)-(CAS)	0.52
Sabinene	0.41
Di-(2-ethylhexyl)phthalate	0.34
Palatambin	0.26
Lvcenin2	0.19
a'-pinene,(−)-	0.17
Isoaromadenolrene poxide	0.15
1,3-Dioxane-4,6-dione,2,2-dimethyl,5,5-bis(2-methyl-2-propenyl)-(CAS)	0.14
2-a'-pinenr	0.14
2 (1H)-Naphthalenones octahydro-aα-methyl-7-(1-methylethyl)-,(4aá,7 a',8a a')-(CAS)	0.12
Camphene	0.1
Total	98.19%

A. flavus and *A. niger* was completely suppressed by fumigation with *C. citratus* at a concentration of 1.5 *μl/ml*. Fumigation with both of *O. majorana* or *O. basilicum* at a concentration of 5.0 *μl/ml* was required to inhibit growth of *A. flavus*. Growth of *A. niger* was completely inhibited by *O. basilicum* essential oil at a concentration of 4.0 *μl* and more than 5.0 *μl* of *O. majorana* essential oil/ml of growth medium. Application of these oils by the contact technique showed that higher concentrations of oils are required to induce suppression. Growth of *A. flavus* and *A. niger* required 2.0 *μl* of *C. citratus* and 10.0 *μl* or more of *O. majorana* essential oils/ml medium. Also higher concentrations of the essential oil of *O. basilicum* were required to inhibit growth of *A. flavus* and *A. niger*, while the former required 5.0 ml/ml the later required 4.0 *μl/ml*.

As for *S. cerevisiae* data indicated that fumigation of Sabouraud's broth medium with 2.0 *μl/ml* of *C. citratus* or 3.0 *μl/ml* of either *O. majorana* or *O. basilicum* completely inhibited growth of *S. cerevisiae*. Meanwhile, 4.0 *μl* of *C. citratus* or 5.0 *μl* of *O. basilicum* or *O. majorana* prevented the growth of the yeast when any of these oils were applied by contact technique.

Table 5. Percentages of components of essential oil extracted from *Origanum majorana*

Component	Percentage
3-cyclohexen-1-01,4-methyl-1(1-methylethyl)-(CAS)	51.5
C-Terpinene	9.2
Trans-caryophyllene	4.4
Sabinene	3.3
Linalyl acetate	2.4
Á-Terpinolene	1.9
Bicyclogermacrene	1.4
Cyclohexane-(1,)-dimethylethyloxy)-2-methyl-(CAS)	1.1
Á-pinene,(–)-	0.73
Spatholenol	0.67
P-Methane-1,2,3,-triol(CAS)	0.57
4-Terpinenyl acetate	0.52
Isospatholenol	0.48
1,2,3-Trihydroxy-p-methane	0.25
Di-(2-ethyl hexyl)phthalate	0.25
Lucenin2	0.2
(E.E)-Farnesylacetone	0.1
Total	78.97%

Resubculture inocula from the three test fungi inhibited by MICs of the three essential oils into nonfumigated media were negative, confirming the fungicidal effect of these concentrations. Also, *C. citratus* essential oil was the highest in its antimicrobial activity than the other two oils, and the sublethal (SLC) dose of this oil (1 *µl*/*ml* medium) when applied by fumigation inhibit 65%, 73% and 97% of *A. flavus, A. niger* and *S. cerevisiae* growth, respectively.

Thermal stability of *C. citratus* essential oil as antifungal agent: For being the highest in antimicrobial activity with a sublethal dose of 1 *µl*/*ml* when applied by fumigation technique *C. citratus* was selected to study its thermal stability. The data presented in Table 7 demonstrate that the percentage of growth inhibition is nearly the same and there is no significant difference between the antifungal activity of autoclaved and nonautoclaved *C. citratus* oil at SLC and MIC against the three test fungi.

Discussion

In the present study, microbial count (CFU/*ml*) of deteriorated juice showed that orange hold higher counts by comparison to guava and banana. Counts of total fungi (Molds and yeasts) were higher than that of bacteria. The increasing in fungal count should be attributed to the high acidic pH values of these fruit juices "3.6~4.3" (Brad-

Table 6. Effect of different concentrations of essential oils on the growth of test organisms

Organism	Concentration of oil *µl*/*ml*	Percentage of growth inhibition*					
		*Cymbopogon citratus***		*Ocimum basilicum*		*Origanum majorana*	
		contact	fumigation	contact	fumigation	contact	fumigation
Aspergillus flavus	0.5	10 ± 2	15 ± 2.6	23 ± 2.6	33 ± 2.6	22 ± 4.4	35 ± 5.6
	1.0	40 ± 1	65 ± 3.6	30 ± 3.6	40 ± 5.6	25 ± 3.6	42 ± 3.6
	1.5	63 ± 5	100	33 ± 2.6	46 ± 3.6	35 ± 4.4	52 ± 1.7
	2.0	100	"	41 ± 6.2	60 ± 6.6	38 ± 5.3	56 ± 4.4
	3.0	"	"	57 ± 5.5	67 ± 5.3	52 ± 2.6	58 ± 5.5
	4.0	"	"	58 ± 3.6	73 ± 3.6	58 ± 3.6	68 ± 5.6
	5.0	"	"	100	100	68 ± 6.2	100
	10.0	"	"	"	"	100	"
Aspergillus niger	0.5	39 ± 4.3	31 ± 4.6	10 ± 3.6	23 ± 3.6	23 ± 4.4	37 ± 5.6
	1.0	43 ± 2.6	73 ± 3.6	23 ± 3.6	35 ± 2.6	25 ± 4.6	58 ± 3.6
	1.5	74 ± 4.6	100	55 ± 7.2	51 ± 3.6	28 ± 4.4	61 ± 1.7
	2.0	100	"	61 ± 6.2	55 ± 7.0	58 ± 5.3	64 ± 4.4
	3.0	"	"	74 ± 4.4	74 ± 4.4	63 ± 2.6	67 ± 5.5
	4.0	"	"	100	100	66 ± 3.6	75 ± 5.6
	5.0	"	"	"	"	72 ± 6.2	86 ± 3.6
	10.0	"	"	"	"	86 ± 4.6	100
Saccharomyces cerevisiae	0.5	71 ± 2	91 ± 2.6	62 ± 2.6	82 ± 2.6	52 ± 4.4	76 ± 5.6
	1.0	80 ± 1	97 ± 3.6	69 ± 3.6	84 ± 5.6	60 ± 3.6	81 ± 3.6
	1.5	88 ± 5	99 ± 3.6	76 ± 2.6	87 ± 3.6	65 ± 4.4	85 ± 1.7
	2.0	93 ± 5	100	84 ± 6.2	90 ± 6.6	70 ± 5.3	91 ± 4.4
	3.0	98 ± 5	"	89 ± 5.5	100	85 ± 2.6	100
	4.0	100	"	93 ± 3.6	"	94 ± 3.6	"
	5.0	"	"	100	"	100	"

The values are mean of three replicates± standard deviation ($P < 0.01$).

A. flavus & *A. niger* grown on Czapek's broth medium pH 5.5 at 28°C for 5 days and *S. cerevisiae* grown on Sabouraud's broth medium pH 6.5 at 37°C for 24 hr.

*For percentage of growth inhibition sees Materials and Methods.

**The results of *Cymbopogon citratus* were previously mentioned by Helal *et al.* (2006a & b; 2007).

Table 7. Effect of autoclaving on the antimicrobial activity of *Cymbopogon citratus* oil fumigated against test organisms

Concentration of oil ($\mu l/ml$)	Percentage of growth inhibition					
	*Aspergillus flavus**		*Aspergillus niger**		*Saccharomyces cerevisiae***	
	non autoclaved	autoclaved	non autoclaved	autoclaved	non autoclaved	autoclaved
1.0	71 + 2.2	68 + 2.0	70 + 3.0	68 + 1.0	98 + 4.0	95 + 3.6
1.5	100	100	100	100	100	98.8

The values are mean of three replicates± standard deviation.
**A. flavus* and *A. niger* grown on Czapek's broth medium pH 5.5 at 28°C for 5 days.
***S. cerevisiae* grown on Sabouraud's broth medium pH 6.5 at 37°C for 24 hr.

dock, 1999; Chen *et al.*, 1993). The most prevalent fungi were species belonging to the genera of *Aspergillus* (4 species), *Penicillium* (2 species) and yeasts (2 species). In a previous study of Hemida (2004) found that many of the preceding species especially *A. niger*, *A. flavus*, *P. digitatum* and *S. cerevisiae* were also predominant among the mycoflora associated with several juices and foodstuffs. Fermentative yeasts, particularly *S. cerevisiae*, are known to be common spoilage agents in refrigerated citrus juices, because of their capacity to produce copious amounts of CO_2 and ethanol (Braddock, 1999; Chen *et al.*, 1993). Essential oils and their constituents are contemporary applied in food preservation and in the manufacture of medicinal antimicrobial agents and disinfectants (Voda *et al.*, 2003). *Botrytis cinerea* is the only organism that failed completely to grow in the presence of any of the eight oils tested (Table 2). Except caraway oil (against *A. flavus*) and fennel oil (against *Paecilomyces lilacinus*), all essential oils tested in the present study showed inhibitory activity against all microorganisms isolated from orange, guava and banana juices. Inhibition of microbial growth by essential oils has been previously recorded. While Romagnoli *et al.* (2005) noticed that *P. digitatum* was inhibited with 1.25 $\mu l/ml$ and *B. cinerea* with 10 $\mu l/ml$ of *Tagetes patula* essential oil, Daferea *et al.* (2000) also noticed that 400 $\mu l/ml$ *Origanum majorana* essential oil totally inhibited the mycelial growth of *P. digitatum*. The same oil inhibited the growth of the common spoilage fungus *A. niger* at the concentration of 10 $\mu l/ml$ broth with 91.5% inhibition effect (Baratta *et al.*, 1998). In the present study, lemongrass oil revealed the greatest potential of antimicrobial activity against all test organisms (10 molds, 2 yeasts and 5 bacteria). This oil was followed by basil, thyme and origanum oils. Dube *et al.* (1989) showed that the essential oil of *Ocimum basilicum* at a concentration of 1.5 ml/l completely suppressed the mycelial growth of 22 species of fungi including the mycotoxin producing strains of *A. flavus* and *A. parasiticus*. In a comprehensive study by Pattnaik *et al.* (1996) the antimicrobial activity of some essential oils against 22 bacterial strains, including Gram-positive cocci and rods, Gram-negative rods and 12 fungi were studied using disc diffusion method. Lemongrass, eucalyptus and peppermint essential oils were found to be effective against all tested bacterial strains. They noticed also that aegle and palmarosa oils inhibited 21 bacteria, patchouli and ageratum oils inhibited 20, and citronella and geranium oils were inhibitory to 15 and 12 strains, respectively. All the test fungal species were inhibited by 7 oils namely aegle, citronella, geranium, lemongrass, orange, palmarosa and patchouli. Eucalyptus and peppermint oils were effective against 11 fungal species. Ageratum oil was inhibitory to only 4 of the test fungi. The minimum inhibitory concentration of eucalyptus, lemongrass, palmarosa and peppermints oils ranged from 0.16 $\mu l/ml$ to 720 $\mu l/ml$ for 18 bacteria and from 0.25 $\mu l/ml$ to 10 $\mu l/ml$ for 12 fungi. A screening of the level of inhibitory activity among 51 essential oils tested by Hili *et al.* (1997) using drop diffusion method, showed that the value varied from 0.3 to 90% of total growth of *Escherichia coli*, *Staphylococcus aureus*, *Schizosaccharomyces pombe*, *Saccharomyces cerevisiae*, *Candida albicans* and *Torulopsis utilis*. In a comparison study, Mejlholm and Dalgaard (2002) observed that Oregano, cinnamon and lemongrass oils possess strong antimicrobial activity by comparison with thyme, clove, bay marjoram, sage and basil oils. They reduced the growth rate of the seafood spoilage microorganism *Photobacterium phosphoreum*. In a study by Guynot *et al.* (2003) showed that out of 17 essential oils tested only seven namely; cinnamon leaf, lemongrass, thyme, bay, clove, peppermint and basil inhibited growth of *Eurotium amstelodami*, *E. herbariorum*, *E. repens*, *E. rubrum*, *A. flavus*, *A. niger* and *P. corylophilum* commonly causing deterioration of bakery products. Nielsen and Rios (2000) have proved that volatile substances from mustard, cinnamon, garlic and clove essential oils were efficient in the control of common bread spoilage fungi. However comparison of the data obtained by different studies is difficult; because of differences in plants extract compositions, in methodologies followed to assess antimicrobial activity and in test microorganisms chosen (Hammer *et al.*, 1999).

Essential oils are natural mixtures of hydrocarbons (terpenes), oxygen-containing (alcohols, aldehydes, ketones, carboxylic acids, ethers, lactones) and sulfur-containing (sulfides, disulfides and trisulfides) organic substances of plant and animal origin (Voda *et al.*, 2003). El-Kady *et al.*

(1993) stated that there is a relationship between the chemical structure of the most abundant compounds in the essential oil and the antimicrobial activity. It would also be worthy to be cited here that the composition of any plant essential oil is influenced by the presence of several factors such as; local, climate, plant species, methodology and experimental conditions (Daferea *et al.*, 2000; Mishra and Dubey, 1994; Prudent *et al*., 1995). These factors may alter the biological and antimicrobial activities of the oils produced (Shu and Lawrence, 1997; Vardar-Ünlü *et al.*, 2003). Distillation time and temperature can also significantly affect the oil constituents (Janssen *et al.*, 1987). The oil chemistry profile obtained from GC-MS analysis of these oils showed that the major components of three tested oils were terpenoids. *Cymbopogon citratus* oil contain e-citral (65.4%), z-citral (3.0%) and a-myrcene (6.7%) *Ocimum basilicum* oil contain bezynen,1-methyl1-4-(2-propenyl) 80%, cineole 6.7% and bisabolene 3.8% in, *Origanum majorana* oil contain 51.5% 3-cyclohexen-1-01,4-methyl-1(1-methylethyl)-(CAS), 9.2% C-terpinene and 4.4% Trans-caryophyllene.. It is well known that terpenoids possess strong antimicrobial activity (Singh *et al*., 2002; Vagi *et al*., 2005). Among these terpenoids, Citral, geraniol and citronellol showed the highest antifungal activities (Viollon and Chaumont, 1994). According to Suhr and Nielsen (2003) the main components of *Cymbopogon citratus* oil are; D-limonene, 3.14%; geranial 4.2%, geranial (citral a), 31.93% and neral (citral b) 45.99%. Chemically, citral is an isomeric mix of geranial and neral, both are well known antimicrobial agents of prominent activity against bacteria and fungi (Guynot *et al.*, 2003; Inouye *et al.*, 2001; Kim *et al.*, 1995). Citral is thought to be responsible for the resistance toward post-harvest fungal infections of lemons (Rodov *et al.*, 1995) and preventing spoilage induced by food borne organisms (Kim *et al.*, 1995). Citral is the main constituent of *Cymbopogon citratus* essential oil in the present study and also in other studies by Hammer *et al.* (1999), Inouye *et al.* (2001) and Friedman *et al.* (2004). The antimicrobial action of *Backhousia citroidora* oil was believed to be directly related to its high citral content (Wilkinson *et al.*, 2003). A great number of components such as; terpinene, cineole, pinene, sabinene recorded in the oils of *Ocimum basilicum* and *Origanum majorana* were also noticed by Christoph *et al.* (2000) and Cox *et al.* (2001) in *Melaleuca alternifolia* (tea tree oil), in *Thymus revolutus* oil by Karaman *et al.* (2001) in *T. x-porlock* and *T. eriocalyx* oils by Rasooli and Abyaneh (2004) and also in *Origanum vulgare* oil by Sahin *et al.* (2004). In the present study linalool (as a component of lemongrass oil) and linalyl acetate (as a component of origanum oil) are present also in *Salvia sclarea* essential oil, both exhibited antifungal activity against *Sclerotinia sclerotiorum*, *Sclerotium cepivorum* and *Fusarium oxysporum* (Pitarokili *et al.*, 2002). It has been concluded that the antimicrobial activity of essential oils can differ from that of their major constituents when tested separately probably due to the presence of synergistic or antagonistic effects resulting from the minor components (Lis-Balchin *et al.*, 1998a, b; Pitarokili *et al.*, 2002).

Aspergillus flavus and *A. niger* are saprophytic molds capable of growing upon a wide range of organic substrates and often cause deterioration of stored food materials (Samson *et al.*, 1995). These species are known to produce mycotoxins notably *A. flavus* (aflatoxin) and *A. niger* (nigragillin, malformins, naphthoquinones and oxalic acids) (Frisvad, 1988; Northolt and Soentoro, 1988; Pitt and Hocking, 1997). They are potentially able to cause mycotic diseases to human and other vertebrates (de Hoog *et al.*, 2000). *S. cerevisiae* is the most common spoilage agent in refrigerated citrus juices (Braddock, 1999; Chen *et al.*, 1993). In the present study growth of these fungi is inhibited by each of the eight essential oils tested (Table 2), especially *C. citratus*, *O. basilicum* and *O. majorana* essential oils. Although the majority of these essential oils are classified as Generally Recognized As Safe (GRAS) (Kabara, 1991), their use in foods as preservatives is often limited due to flavor concentrations, since effective antimicrobial doses may exceed organoleptically acceptable levels. Therefore, there is an increasing demand for accurate knowledge of the minimum inhibitory (effective) concentrations (MIC) of essential oils to enable a balance between the sensory acceptability and antimicrobial efficacy. This could be achieved *in vitro* using dilution method which provides more quantitative results as recommended by Manou *et al.* (1998). The present data showed that *C. citratus* essential oil appear to be more toxic than that of *O. basilicum* and *O. majorana* essential oils against *A. flavus*, *A. niger* and *S. cerevisiae*. It caused complete growth inhibition of the three fungi at 1.5 or 2.0 *μl/ml* medium when applied by fumigation or contact methods, respectively. i.e., the MIC in case of fumigation method is less than that when the oils applied by contact method in the medium. Mishra and Dubey (1994) recorded that lemongrass oil exhibited a broad spectrum of fungitoxicity by inhibiting the growth of 35, 45 and 47 fungal species, which cause deterioration of stored food commodities including *A. flavus* and *A. niger*. This oil was also found to be the second highest active as anti *Trichophyton* among seven oils namely; cinnamon bark, thyme, perilla, lavender, tea tree and citron essential oils. The antifungal activity of these oils against *Trichophyton mentagrophytes* and *T. rubrum* was more enhanced by vapor action than solution contact (Inouye *et al.*, 2000, 2001). These authors suggested that this might be caused by the combined effect of vapor action on mycelia or spores and action after absorption on agar. The different MIC values obtained for each of the three essential oils applied by fumigation or contact methods show that the level of antimicrobial activity of essential oils is closely dependent on the screening method used (Delespaul *et al.*, 2000). It has been reported that the antifungal effect of essential oils is dependent on the application method, for example, larger phenolic compounds such as thymol and eugenol (Thyme, cinnamon and clove) had the best effect when applied directly to the medium, whereas smaller compounds such as allyl isothiocyanate and citral (mustard and lemongrass) were most efficient when added as volatiles (Suhr and Nielsen, 2003).

Data obtained in the present study, showed that *C. citratus* essential oil was not affected when preautoclaved at 121°C for 30 min. This thermostable nature of the oil fungitoxicity was previously reported by Mishra and Dubey (1994) at concentrations of 1000 and 1500 ppm after 5~100°C treatments. They added that this oil was also non phytotoxic, exhibited no animal toxicity, more efficacious than 10 synthetic fungicides and its potency is not affected by increasing the density of the inoculums of the tested *A. flavus*. These advantages and others such as antitoxic property (Helal *et al*., 2007) and antioxidant activity (Helal *et al.* unpublished data) increase the possibility of using *C. citratus* essential oil for juice preservation in future studies.

References

Ali, N. M., Hohtar, M., Shaari, K., Rahmanii, M., Ali, A. M. and Jantan, I. 2002. Chemical composition and antimicrobial activities of the essential oils of *Cinnamomum aureofulvum* Gamb. *J. Essent. Oil Res*. **14**: 135-138.

Alviano, W. S., Mendonca-Filho, R. R., Alviano, D. S., Bizzo, H. R., Souto-Padron, T., Rodrigues, M. L., Bolognese, A. M., Alviano, C. S. and Souza, M. M. G. 2005. Antimicrobial activity of *Croton cajucara* Benth linalool-rich essential oil on artificial biofilms and planktonic microorganisms. *Oral Microbiol. Immunol*. **20**: 101-105.

Bankole, S. A. and Adebanjo, A. 1995. Inhibition of growth of some plant pathogenic fungi using some Nigerian plants. *Int. J. Tropical Plant Diseases* **13**: 91-95.

Baratta, M. T., Dorman, H. J. D., Deans, S. G., Figueiredo, A. C., Barroso, J. G. and Ruberto, G. 1998. Antimicrobial and antioxidant properties of some commercial essential oils. *Flavour and Fragrance J.* **13**: 235-244.

Barnett, J. A., Payne, R. W. and Yarrow, D. 2000. Yeasts: characteristics and identification. 3rd ed. Cambridge Uni. Press. Cambridge, U.K.

Bishop, C. D. and Thornton, I. B. 1997. Evaluation of the antifungal activity of the essential oils of *Monarda citriodora* var. *citriodora* and *Melaleuca alternifolia* on post-harvest pathogens. *J. Essent. Oil Res*. **9**: 77-82.

Braddock, R. J. 1999. Single strength orange juices and concentrate. Pp 53-83. *In*: Handbook of citrus by products and processing technology. John Wiley & Sons, Inc., New York.

Carson, C. F. and Riley, T. V. 1995. Antimicrobial activity of the major components of the essential oil of *Melaleuca alternifolia. J. Appl. Bacteriol*. **78**: 264-269.

Chen, C. S. P., Shaw, P. E. and Parish, M. E. 1993. Orange and tangerine juices. Pp 110-165. *In*: Nagy, C. S. Chen, and P. E. Shaw. Eds. Fruit juice processing technology. Agscience, Auburndale, Fla.

Christoph, F., Kaulfers, P. M. and Stahl-Biskup, E. 2000. A comparative study of the *in vitro* antimicrobial activity of tea tree oils with special reference to the activity of B-Triketones. *Planta Med*. **66**: 556-560.

Cox, S. D., Mann, C. M. and Markham, J. L. 2001. Interactions between components of the essential oil of *Melaleuca alternifolia*. *J. Appl. Microbiol*. **91**: 492-497.

Daferea, D. J., Ziogas, B. N. and Polissiou, M. G. 2000. GC-MS analysis of essential oils from some Greek aromatic plants and their fungitoxicity on *Penicillium digitatum*. *J. Agric. Food Chem*. **48**: 2576-2581.

de Hoog, G. S., Guarro, J., Gene, J. and Figueras, M. J. 2000. Atlas of clinical fungi, 2nd ed. CBSU, The Netherlands.

Delaquis, P. J., Stanich, K., Girard, B. and Mazza, G. 2002. Antimicrobial activity of individual and mixed fractions of dill, cilantro, coriander and eucalyptus essential oils. *Int. J. Food Microbiol*. **74**: 101-109.

Delespaul, Q., Debillerbeck, V. G., Roques, C. G., Michel, G., Marquier-Vinuales, C. and Bessiere, J. M. 2000. The antifungal activity of essential oils as determined by different screening methods. *J. Essent. Oil Res*. **12**: 256-266.

Domsch, K. H., Gams, W. and Anderson, T. 1980. Compendium of soil fungi. Academic Press, London and New York.

Dube, S., Upadhyay, P. D. and Tripathi, S. C. 1989. Antifungal, physicochemical and insect repelling activity of the essential oil of *Ocimum basilicum*. *Can. J. Bot*. **67**: 2085-2087.

El-Kabouss, A., Charrouf, Z., Faid, M., Garneau, F. and Collin, G. 2002. Chemical composition and antimicrobial activity of the leaf essential oil *Argania spinosa* L. *Skeels. J. Essent. Oil Res.* **14**: 147- 149.

El-Kady, I. A., El-Maraghy, S. S. M. and Mostafa, E. M. 1993. Antibacterial and antidermatophyte activities of some essential oils from spices. *Qatar Univ. Sci. J.* **13**: 63-69.

El-Kamali, H. H., Ahmed, A. H., Mohamed, A. S., Yehia, A. A. M., El-Tayeb, I. and Ali, A. A. 1998. Antibacterial properties of essential oils from *Nigella sativa* seeds, *Cymbopogon citratus* leaves and *Pulicaria undulata* aerial parts. *Fitoterapia* **69**: 77-78.

Friedman, M., Henika, P. R., Levin, C. E. and Mandrell, R. E. 2004. Antibacterial activities of plant essential oils and their components against *Escherichia coli* 0157:H7 and *Salmonella enterica* in apple juice. *J. Agric. Food Chem*. **52**: 6042-6048.

Frisvad, J. C. 1988. Fungal species and their specific production of mycotoxins. Pp 239-249. *In*: Sanson, R.A. & Van Reenen-Hoekstra. Eds. Introduction to Foodborne Fungi. Centraalbureau voor Schimmelcultures, Baarn, The Netherlands.

Gams, W., Hoekstora, E. S. and Aptroot, A. 1998. CBS course of mycology. 4th ed. Centraalbureau Voor Schimmelcultures, Baarn, The Netherlands.

Gowda, N. K. S., Malathi, R. U. and Suganthi, R. U. 2004. Effect of some chemical and herbal compounds on growth of *Aspergillus parasiticus* and aflatoxin production. *Animal Feed Sci. Tech*. **116**: 281-291.

Guynot, M. E., Ramos, A. J., Seto, L., Purroy, P., Sanchis, V. and Marin, S. 2003. Antifungal activity of volatile compounds generated by essential oils against fungi commonly causing deterioration of bakery products. *J. Appl. Microbiol*. **94**: 893-899.

Hammer, K. A., Carson, C. F. and Riley T. V. 1999. Antimicrobial activity of essential oils and other plant extracts. *J. Appl. Microbiol*. **86**: 985-990.

Hemida, S. K. 2004. Influence of some commercial oils on growth of fungi and their degrading enzymes. *Egypt. J. Biomed. Sci*. **16**: 365-380.

Helal, G. A., Sarhan, M. M., Abu Shahla, A. N. K. and Abou El-Khair, E. M. 2006a. Effect of *Cymbopogon citratus* L. essential oil on the growth and morphogenesis of *Saccharomyces cerevisiae* ML2 strain. *J. Basic Microbiol*. **46**: 375-386.

Helal, G. A., Sarhan, M. M., Abu Shahla, A. N. K. and Abou El-Khair, E. M. 2006b. Effect of *Cymbopogon citratus* L. essential oil on the growth and morphogenesis of *Aspergillus niger* ML2 strain. *J. Basic Microbiol*. **46**: 456-469-386.

Helal, G. A., Sarhan, M. M., Abu Shahla, A. N. K. and Abou El-Khair, E. M. 2007. Effect of *Cymbopogon citratus* L. essential oil on the growth, morphogenesis and aflatoxin production of *Aspergillus flavus* ML2 strain. *J. Basic Microbiol*. **47**(in press).

Hili, P., Evans, C. S. and Veness, R. G. 1997. Antimicrobial action of essential oils: the effect of dimethylsulphoxide on the activity of cinnamon oil. *Lett. Appl. Microbiol*. **24**: 269-275.

Holt, J. G., Sneath, P. H. A., Mair, M. S. and Sharpee, M. E. 1986. Bergey's Manual of systematic bacteriology, vol. 2. Williams & Wilkins, 428 east Preston Street, Baltemore, MD 211202, U.S.A.

Inouye, S., Tsuruoka, M., Watanabe, M., Takeo, K., Akao, M., Nishiyama, Y. and Yamaguchi, H. 2000. Inhibitory effect of essential oils on apical growth of *Aspergillus fumigatus* by vapour contact. *Mycoses* **43**: 17-23.

Inouye, S., Tsuruoka, T., Uchida, K. and Yamaguchi, H. 2001. Effect of sealing and Tween 80 on the antifungal susceptibility testing of essential oils. *Microbiol. Immunol*. **43**: 201-208.

Janssen, A. M., Scheffer, J. J. C. and Baerheim Svendeaen, A. 1987. Antimicrobial activity of essential oils: a 1976-86 literature review. Aspects of the test methods. *Planta Med*. **53**: 395-398.

Johnson, L. F., Curl, E. A., Bond, J. H. and Fribourg, H. A. 1959. Methods for studying soil microflora-plant diseases relationships. Burgess, Minneqpolis.

Kabara, J. J. 1991. Phenols and chelators. Pp 200-214. *In*: Russell, N. J. and Gould, G. W. Eds. *Food Preservatives*. London: Blackie.

Karaman, S., Digrak, M., Ravid, V. and Ilcim, A. 2001. Antibacterial and antifungal activity of the essential oils of *Thymus revolutus* Celak from Turkey. *J. Ethnopharmacol*. **76**: 183-186.

Kim, J., Marshall, M. R. and Wei, C. 1995. Antibacterial activity of some essential oil components against five food borne pathogens. *J. Agric. Food Chem*. **43**: 2839-2845.

Kitch, M. A. and Pitt, J. I. 1992. A laboratory guide to the common *Aspergillus* species and their Teleomorphs published by common wealth scientific and Industrial Research Organization. Division of Food Processing (CSIRO), Sydney.

Krauze-Baranowska, M., Mardarowicz, M., Wiwart, M., Poblocka, L. and Dynowska, M. 2002. Antifungal activity of the essential oils from some species of the genus *Pinus*. *Z. Naturforsch*. **572**: 478- 482.

Lis-Balchin, M., Buchbauer, G., Hirtenlehner, T. and Resch, M. 1998a. Antimicrobial activity of *Pelargonium* essential oils added to a quiche filling a model food system. *Lett. Appl. Microbiol*. **27**: 207-210.

Lis-Balchin, M., Deans, S. G. and Eaglesham, E. 1998b. Relationship between bioactivity and chemical composition of commercial essential oils. *Flavour Fragrance J*. **13**: 98-104.

Manou, L., Bouillard, L., Devleeschouwer, M. J. and Barel, A. O. 1998. Evaluation of the preservative properties of *Thymus vulgaris* essential oil in topically applied formulations under a challenge test. *J. Appl. Microbiol*. **84**: 368-376.

Mejlholm, O. and Dalgaard, P. 2002. Antimicrobial effect of essential oils on the seafood spoilage microorganism *Photobacterium phosphoreum* in liquid media and fish products. *Lett. Appl. Microbiol*. **34**: 27-31.

Mishra, A. K. and Dubey, N. K. 1994. Evaluation of some essential oils for their toxicity against fungi causing deterioration of stored food commodities. *Appl. Environ. Microbiol*. **60**: 1101-1105.

Moubasher, A. H. 1993. Soil fungi in Qatar and other Arab countries. Published by the centre for scientific and applied research. University of Qatar. The Doha Modern Printing Press.

Nielsen, P. V. and Rios, R. 2000. Inhibition of fungal growth on bread by volatile components from spices and herbs and the possible application in active packaging, with special emphasis on mustard essential oil. *Int. J. Food Microbiol*. **60**: 219-229.

Northolt, M. D. and Soentoro, P. S. S. 1988. Fungal growth on foodstuffs related to mycotoxin contamination. Pp 231-238. *In*: Samson, R. A. and Van Reenen-Hoekstra. Eds. Introduction to Foodborne Fungi. Centraalbureau voor Schimmelculures, Barrn, The Netherlands.

Pattnaik, S., Subramanyam, V. R. and Kole, C. 1996. Antibacterial and Antifungal activity of ten essential oils *in vitro*. *Microbios* **86**: 237-246.

Pitarokili, D., Couladis, M., Petsikos-Panayotarou, N. and Tzakou, O. 2002. Composition and antifungal activity on soil-borne pathogens of the essential oil of *Salvia sclarea* from Greece. *J. Agric. Food Chem*. **50**: 6688-6691.

Pitt, J. I. 1979. The genus *Penicillium* and its teleomorphic states *Eupenicilium* and *Talaromyces*. Academic Press, London and New York.

Pitt, J. I. 1986. A laboratory guide to common *Penicillium* species. Commonwealth scientific and Industrial Research Organization (CSIRO). Division of Food Processing, Sydney.

Pitt, J. I. and Hocking, A. D. 1997. Fungi and food spoilage. Blackie Academic and Professional, London.

Prudent, D., Perineau, F., Bessiere, J. M., Michel, G. M. and Baccou, J. C. 1995. Analysis of the essential oil of wild oregano form Martinique (*Coleus aromaticus* Benth.). Evaluation of its bacteriostatic and fungistatic properties. *J. Essent. Oil Res*. **7**: 165-173.

Raper, K. B. and Fennell, D. I. 1977. The genus *Aspergillus* Robert E. Krieger Publishing Company. Huntington, New York.

Raper, K. B. and Thom, C. 1968. A manual of the *Penicillium*. Hafner Publishing Company, New York and London.

Rasooli, I. and Abyaneh, M. R. 2004. Inhibitory effects of Thyme oils on growth and aflatoxin production by *Aspergillus parasiticus*. *Food Control* **15**: 479-483.

Rodov, V., Ben-Yehoshua, S. and Fang, D. Q. 1995. Ashkenazi preformed antifungal compounds of lemon fruit: citral and its relation to disease resistance, *J. Agric. Food Chem*. **43**: 1057-1061.

Romagnoli, C. Bruni, R., Andreotti, E., Rai, M. K., Vicentini, C.

B. and Mares, D. 2005. Chemical characterization and antifungal activity of essential oil of capitula from wild Indian *Tagetes patula* L. *Protoplasma* **225**: 57-65.

Sahin, F., Gulluce, M. M., Daferera, D., Sokmen, A., Sokmen, M., Polissiou, M., Agar, C. and Ozer, H. 2004. Biological activities of the essential oils and methanol extract of *origanum vulgare* ssp. *Vulgare* in the eastern Anatolia region of Turkey. *Food Control* **15**: 549-557.

Samson, A. R., Hoekstra, E. S., Frisvad, J. C. and Filtenborg, O. 1995. Introduction to food-borne fungi. CBS. The Netherlands, p 64.

Sechi, L. A., Lezcano, I., Nunez, N., Espim, M., Dupre, I., Pinna, A., Molicotti, P., Fadda, G. and Zanetti, S. 2001. Antibacterial activity of ozouized sunflower oil (Oleozon). *J. Appl. Microbiol.* **90**: 279- 284.

Shadab, O., Hanif, M. and Chaudhary, F. M. 1992. Antifungal activity by lemongrass essential oils. *Pakistan J. Sci. Int. Res.* **35**: 246-249.

Shu, C. K. and Lawrence, B. M. 1997. Reasons for the variation in composition of some commercial essential oils. Pp 138-159. *In*: S. J. Risch and C. T. Ho. Eds. *ACS symposium series*: Col.660. Spices, Flavor chemistry and antioxidant properties.

Singh, G., Singh, O. P. and Maurya, S. 2002. Chemical and biocidal investigations on essential oils of some Indian *Curcuma* species. *Progress in Crystal Growth and Characterization of Materials* **45**: 75-81.

Suhr, K. I. and Nielsen, P. V. 2003. Antifungal activity of essential oils evaluated by two different application techniques against rye bread spoilage fungi. *J. Appl. Microbiol.* **94**: 665-674.

Thompson, D. P. 1989. Fungitoxic activity of essential oil components on food storage fungi. *Mycologia* **81**: 151-153.

Vagi, E., Simandi, B., Suhajda, A. and Hethelyi, E. 2005. Essential oil composition and antimicrobial activity of *Origanum majorana* L. extracts obtained with ethyl alcohol and supercritical carbon dioxide. *Food Res. Int.* **38**: 51-57.

Vardar-Ünlü, G., Candan, F., Sokmen, A., Daferera, D., Polissiou, M., Sokmen, M., Donmez, E. and Tepe, B. 2003. Antibacterial and antioxidant activity of the essential oil and methanol extracts of *Thymus pectinatus* Fisch. et Mey var. Pectinatus (Lamiaceae). *J. Agric. Food Chem.* **51**: 63-67.

Viollon, C. and Chaumont, J. P. 1994. Antifungal properties of essential oils and their main components upon *Cryptococcus neoformans*. *Mycopathologia* **128**: 151-153.

Voda, K., Boh, B., Vrtacnik, M. and Pohleven, F. 2003. Effect of the antifungal activity of oxygenated aromatic essential oil compounds on the white-rot *Trametes versicolor* and the brown-rot *Coniophora puteana. Int. Biodeterior. Biodegrad.* **51**: 51-59.

Wilkinson, J. M., Hipwell, M., Ryan, T. and Cavanagh, H. M. A. 2003. Bioactivity of *Backhousia citriodora*: Antibacterial and Antifungal activity. *J. Agric. Food Chem.* **51**: 76-81.