J. Pest Cont. & Environ. Sci. 16 (1/2): 69 – 86 (2008).

In vitro inhibition of plant pathogenic fungi and control of gray mold and soft rot of strawberry by essential oils

Soad M. Ahmed and Samir A. M. Abdelgaleil
Pesticides Chemistry Department, Faculty of Agriculture, 21545-El-Shatby, Alexandria University, Alexandria, Egypt

ABSTRACT

The essential oils of eight plants, *Artemisia judaica*, *Achillea santolina*, *Eucalyptus camaldulensis*, *E. citriodora*, *E. smithii*, *Lantana camara*, *Majorana hortensis* and *Mentha microphylla*, were obtained by hydrodistillation and steam distillation. The isolated oils were tested for their *in vitro* antifungal activity against ten phytopathogenic fungi; *Alternaria alternata*, *Botrytis cinerea*, *B. fabae*, *Fusarium culmorum*, *F. oxysporum*, *Helminthosporium sp.*, *Penicillium digitatum*, *Rhizoctonia solani*, *Rhizopus stolonifer* and *Sclerotium rolfsii*. Five of the isolated oils were evaluated for the control of postharvest decay in strawberry caused by *B. cinerea* and *R. stolonifer*. In an *in vitro* assay, the oil of *M. microphylla* caused complete growth inhibition of *F. culmorum*, *F. oxysporum*, *P. digitatum*, *R. solani* and *R. stolonifer* at a concentration of 500 mg/L. The oils of *A. judaica* and *E. citriodora* also showed complete inhibition of *R. solani* at the same concentration. Comparative antifungal activity revealed that the oil of *M. microphylla* was the most effective, followed by *E. camaldulensis* and *A. judaica*. When tested for the control of gray mold and soft rot in strawberry, the essential oils exhibited a strong reduction of disease incidences. The oils of *E. camaldulensis*, *A. judaica* and *A. santolina* were the most effective in the control of both gray mold and soft rot diseases. To correlate the antifungal activities with chemical compositions of the isolated oils, GC-MS analysis of the tested oils was conducted. The dominant components of the oils consisted of four main groups of oxygenated monoterpenes, monoterpene hydrocarbons, sesquiterpene hydrocarbons and oxygenated sesquiterpenes. The remarkable *in vitro* and *in vivo* antifungal activity of the oils of *E. camaldulensis*, *A. judaica* and *A. santolina* reported in this study indicated that these oils may provide an alternative mean for controlling postharvest pathogens.

Keywords: Essential oils, chemical composition, plant pathogenic fungi, antifungal activity, strawberry

INTRODUCTION

The growing interest in the substitution of conventional fungicides by natural ones has fostered research on the screening of plant materials in order to identify new components with potential fungicidal properties. The synthetic chemicals used nowadays in fungi control may be phased out in near future due to their potential adverse impact on the environment and the development of fungi resistance (Brent and Hollomon, 1998). Therefore, the biodegradable alternatives should be developed worldwide for reducing pre- and post-harvest losses.

Essential oils are a complex mixture of compounds, mainly monoterpenes, sesquiterpenes, and their oxygenated derivatives (alcohols, aldehydes, esters, ethers, ketones, phenols and oxides). Other volatile compounds include phenylpropenes and specific sulphur- or nitrogen-containing substances. Generally, the oil composition is a balance of various compounds, although in many species one constituent may prevail over all others (Cowan, 1999).

There are enormous studies on the antimicrobial activity of plant essential oils against human pathogenic fungi and bacteria, and food borne bacteria (Burt, 2004). However, few studies have been reported on the antifungal activity of essential oils against plant pathogenic fungi. For examples, the essential oil of *Metasequia glyptostroboides* showed antifungal activity against seven plant pathogenic fungi (Bajpai *et al.,* 2007). The essential oils of *Achillea millefolium*, *Cymbopogon citrates*, *Eucalyptus citriodora* and *Ageratum conyzoides* displayed fungitoxicity on the fungus *Didymella bryoniae* (Fiori *et al.,* 2000). The vapour phase of spearmint, tea tree, pine and cinnamon oils controlled the growth of eight common postharvest fungal pathogens growing *in vitro* (Szczerbanik *et al.,* 2007). It has been reported that 4 eucalyptus species, *Eucalyptus urophylla*, *E. grandis*, *E. camaldulensis*, and *E. citriodora* possessed antifungal activity against seven mildew fungi (Su *et al.,* 2006). Lee *et al.* (2007) stated that 25 essential oils had fumigant activity against post-harvest pathogens *Botrytis cinerea* and *Colletotrichum gloeosporioides*.

The purpose of this study was; 1) to test the *in vitro* fungitoxic effect of the essential oils of eight plants namely, *Artemisia judaica*, *Achillea santolina*, *Eucalyptus camaldulensis*, *E. citriodora*, *E. smithii*, *Lantana camara*, *Majorana hortensis* and *Mentha microphylla* against ten plant pathogenic fungi: *Alternaria alternata*, *Botrytis cinerea*, *B. fabae*, *Fusarium*

culmorum, *F. oxysporum*, *Helminthosporium sp.*, *Penicillium digitatum*, *Rhizoctonia solani*, *Rhizopus stolonifer* and *Sclerotium rolfsii*, 2) to evaluate the potential application of the essential oils to control two of the most economic postharvest diseases, gray mold and soft rot, on strawberries, 3) to correlate the antifungal activity of these oils with their chemical profiles.

MATERIALS AND METHODS

Fungi: The following ten phytopathogenic fungi species were used: *Alternaria alternata* (Fr.) Keissl., *Botrytis cinerea* (Pers.), *Botrytis fabae* (Sardina), *Fusarium culmorum* (Smith) Sacc., *Fusarium oxysporum* (Schltdl.), *Helminthosporium sp.*, *Penicillium digitatum* (Pers.) Sacc., *Rhizoctonia solani* (Kuhn), *Rhizopus stolonifer* (Ehrenb.: Fr.) Vuill. and *Sclerotium rolfsii* (Sacc.). The fungi were maintained during the course of the experiments on Potato Dextrose Agar (PDA).

Plant materials: Various parts of six plant species: *Artemisia judaica* L. (aerial parts), *Achillea santolina* L. (aerial parts), *Eucalyptus camaldulensis* Dehnh. (leaves), *Lantana camara* L. (leaves), *Majorana hortensis* Moench (leaves) and *Mentha microphylla* C. Koch. (leaves) were collected during the flowering stage from different locations of Alexandria Governorate and Sinai Peninsula**,** Egypt, in August, 2006 and April, 2007. The plant materials were identified with guidance of the Student's Flora of Egypt (Tackholm, 1974).

Extraction of essential oil: The plant materials were dried at room temperature (26±1ºC) for five days. Essential oils were extracted by hydrodistillation in a Clevenger-type apparatus for 2 h. The oils were dried over anhydrous sodium sulfate, and stored at 4°C until used for biological activities and GC-MS analysis. The oils of two *Eucalyptus* trees (*E. citriodora* and *E. smithii*) grown in Brazil, extracted by steam distillation of fresh leaves, were obtained from Distillery Tres Barras Company, Torrinha city, Sao Paulo, Brazil.

Analysis of essential oils: Essential oils were diluted in diethyl ether and 1 µl was injected into a gas chromatograph (TRACE GC 2000, THERMO) /mass spectrometry (SSQ 7000, FINNIGAN) (GC/MS) set-up. GC-MS analysis was curried out at the National Research Center, Cairo. The GC

column was a 60m (0.25mm i.d.) DB-5 (5%-Phenyl) Methylpolysiloxane capillary column. The GC conditions were as follows: injector temperature, 220ºC; column temperature, isothermal at 40ºC for 2 min, then programmed to 250ºC at 5ºC/2 min and held at this temperature for 2 min; ion source temperature, 200ºC. Helium was used as the carrier gas at the rate of 1 ml/min. The effluent of the GC column was introduced directly into the ion source of the MS. Spectra were obtained in the EI mode with 70 eV ionization energy. The sector mass analyzer was set to scan from 40 to 400 amu for 5 s.

***In vitro* antifungal assay:** The antifungal activity of the isolated essential oils was tested using the mycelial radial growth technique (Zambonelli *et al.,* 1996). First, the eight essential oils were tested against the ten tested fungi at concentration of 500 mg/l. Oils caused mycelial growth inhibition greater than 50% were further evaluated at a series of concentration of 0.05, 0.1, 1, 5, 10, 25, 50, 100, 200, 300, 400, 500 mg/l. Appropriate volumes of the stock solutions of the essential oils in dimethyl sulfoxide (DMSO) were added to Potato Dextrose Agar (PDA) medium immediately before pouring into the Petri dishes (9.0 cm in diameter) at 40-45ºC. Each concentration was tested in triplicate. Parallel controls were maintained with DMSO mixed with PDA. The discs of mycelial felt (0.5 cm diameter) of the plant pathogenic fungi, taken from 8-day-old cultures on PDA plates, were transferred aseptically to the centre of Petri dishes. Thiophanate-methyl (99.9%; Nippon Soda Co., Japan), a wide spectrum fungicide, was used as a reference. The treatments were incubated at 25ºC in the dark. Colony growth diameter was measured after the fungal growth in the control treatment had almost completely covered the Petri dishes. Percentage of mycelial growth inhibition was calculated from the formula: Mycelial growth inhibition (%) = [(DC-DT) /DC] × 100 (Pandy *et al.,* 1982), where DC and DT are average diameters of fungal colony of control and treatment, respectively.The concentration of the essential oil that inhibiting the fungi mycelial growth by 50% (EC_{50}) was determined by a linear regression method (Finney, 1971).

***In vivo* antifungal assay:** The two fungi *R. stolonifer and B. cinerea* were isolated from infected strawberry. The isolated fungi were cultured on Potato Dextrose Agar (PDA). Conidia of fungi were recovered from cultures of 10–14 days old by adding 10 ml of sterile water to each plate. The mycelial suspension was filtered through three layers of sterile cheese cloth. Conidia were counted using Hemacytometer and the concentration of the

conidial suspension was adjusted to 1 x 10^6 conidia / ml. Strawberry fruits (*Fragaria ananassa* cv. Aromas) were obtained from a farm in Elhamam region, west Alexandria city. The berries of uniform size, free of physical damage and visible fungal infection were selected. Fruits were surface sterilized with 70% ethanol, followed by washing three times with distilled water. Three wounds (2-mm diameter and 2-mm deep) were made on the surface of one side of each fruit using a sterile cork borer. The fruit were dipped in water solutions of tested oils containing 0.05% Tween 80 at concentrations 250, 500, 1000 and 2000 mg/l for 1 min. The fruit were left to dry at room temperature. Control fruits were dipped in distilled water containing 0.05% Tween 80. Thiabendazole (Sigma, USA), a reference fungicide, commonly used in Egypt for the control postharvest diseases in strawberry, was tested at the same concentrations for comparison. The fruits were inoculated by applying 10 µl of conidial suspension of *B. cinerea* and *R. stolonifer* on each wound. The inoculated fruits were kept in closed plastic trays to maintain an adequate relative humidity and temperature. Each treatment was replicated four times with 9 fruits per replicate. The number of infected fruits was daily recorded. Disease incidence (the percentage of fruit with visible disease development) was calculated after three days of incubation. A fruit was classified as infected when rot extended more than 1mm beyond the inoculated wound.

Statistical analysis: Mycelial growth inhibition and disease incidence were subjected to one-way analysis of variance (ANOVA) followed by Student-Newman-Keuls test (Cohort software Inc., 1985) to determine the significant differences among mean values at the probability level of 0.05.

RESULTS

***In vitro* antifungal activities of the isolated essential oils:** In the preliminary, test the eight essential oils were tested against the ten tested fungi at a concentration of 500 mg/l. The results of this experiment are shown in Table (1). Some of the tested oils revealed a potent antifungal activity with 100 % mycelial growth inhibition. For examples, the oil of *M. microphylla* caused complete growth inhibition of *F. culmorum*, *F. oxysporum*, *P. digitatum*, *R. solani* and *R. stolonifer*. Similarly the oils of *A. judaica* and *E. citriodora* showed complete inhibition on *R. solani*. On the other hand, some of the tested oils showed a strong antifungal activity with

mycelial growth inhibition greater than 50% and others displayed a weak activity with mycelial growth inhibition ranged from 0 to less than 50%. In general, the oil of *M. microphylla* was the most potent against all of tested fungi followed by *E. camaldulensis* and *A. judaica*, while the oils of *M. hortensis*, *E. smithii* and *E. citriodora* were the least in this respect.

The oils which caused mycelial growth inhibition greater than 50% at tested concentration (500 mg/l) were further evaluated to determine the concentration of the essential oil that inhibiting the mycelial growth by 50% (EC_{50}). Table (2) represents the EC_{50} values, 95% Confidence limits and other regression analysis parameters of some isolated essential oils and the reference fungicide (thiophanate methyl) against the tested fungi. The oil of *M. microphylla* was the only oil which displayed antifungal activity against the ten tested fungi with EC_{50} values less than 500 mg/l.

This oil showed the highest antifungal activity against the fungus *P. digitatum* (EC_{50} = 63.50 mg/l) followed by *R. stolonifer* (EC_{50} = 174.82 mg/l), *R. solani* (EC_{50} = 200.27 mg/l) and *F. culmorium* (EC_{50} = 205.40 mg/l). In addition, the oil of *M. microphylla* had a moderate antifungal activity against *S. rolfsii*, *F. oxysporum*, *Helminthosporium sp.*, *B. fabae* and *A. alternata* and a weak activity against *B. cinerea*. The second most effective oil among the tested oils was *E. camaldulensis* oil which showed antifungal activity against seven of the ten tested fungi. This oil revealed the strongest antifungal activity against the fungus *P. digitatum* with EC_{50} value of 85.50 mg/l, while presented a moderate to a weak activity against the rest of fungi. The oil of *A. judaica* displayed antifungal activity against six of the ten tested fungi. This oil was the most effective one among all of tested oils against the fungus *R. stolonifer* with EC_{50} value of 59.77 mg/l. The oils of *A. santolina* and *L. camara* showed antifungal activity against four and three fungi, respectively, while that of *E. citriodora* was only active against *R. solani*. The later oil was the most effective oil against this fungus with EC_{50} value of 129.68 mg/l. The oils *E. smithii* and *M. hortensis* revealed a very weak antifungal activity against all tested fungi with EC_{50} values more than 500 mg/l.

Table (1). Percentages of mycelial growth of plant pathogenic fungi treated with the extracted essential oils at 500 mg/l

Oil	Percentages of mycelial growth inhibition (± S.D.)									
	Aa	Bc	Bf	Fc	Fo	Hs	Pd	Rso	Rst	Sr
Artemisia judaica	59.4^{bc} (± 1.10)	47.1^{b} (± 2.97)	42.5^{c} (± 0.58)	66.2^{b} (± 4.07)	0.0^{d} (± 0.0)	57.9^{b} (± 1.39)	78.3^{c} (± 3.84)	100.0^{a} (± 0.0)	95.2^{a} (± 4.10)	25.0^{d} (± 0.0)
Achillea santolina	55.6^{c} (± 2.71)	28.6^{c} (± 0.0)	32.5^{d} (± 1.33)	62.7^{b} (± 2.77)	8.3^{c} (± 0.0)	28.3^{d} (± 0.58)	60.3^{d} (± 3.67)	13.0^{e} (± 1.47)	62.5^{b} (± 6.09)	31.7^{c} (± 2.89)
Eucalyptus camaldulensis	96.2^{a} (± 6.58)	29.4^{c} (± 3.18)	62.1^{b} (± 1.99)	53.7^{c} (± 9.37)	33.7^{b} (± 0.64)	55.1^{b} (± 2.19)	85.7^{b} (± 0.0)	75.9^{b} (± 4.52)	63.8^{b} (± 1.90)	44.4^{b} (± 4.79)
Eucalyptus citriodora	31.0^{c} (± 4.10)	26.2^{c} (± 4.16)	0.0^{e} (± 0.0)	30.0^{d} (± 4.66)	13.0^{c} (± 4.28)	37.0^{c} (± 8.23)	0.0^{f} (± 0.0)	100.0^{a} (± 0.0)	0.0^{e} (± 0.0)	12.0^{e} (± 3.25)
Eucalyptus smithii	8.4^{f} (± 3.48)	0.0^{e} (± 0.0)	36.5^{d} (± 3.67)	9.5^{e} (± 2.40)	11.9^{e} (± 8.26)	17.1^{e} (± 4.97)	0.0^{f} (± 0.0)	28.1^{d} (± 2.19)	0.0^{e} (± 0.0)	12.9^{e} (± 0.81)
Lantana camara	59.4^{bc} (± 6.27)	0.0^{e} (± 0.0)	0.0^{e} (± 0.0)	26.7^{d} (± 1.90)	0.0^{d} (± 0.0)	55.0^{b} (± 3.91)	28.6^{e} (± 3.91)	48.9^{c} (± 6.10)	54.8^{c} (± 1.97)	0.0^{f} (± 0.0)
Majorana hortensis	43.8^{d} (±0.95)	28.3^{c} (±0.58)	0.0^{e} (± 0.0)	0.0^{f} (± 0.0)	0.0^{d} (± 0.0)	14.3^{e} (± 0.0)	0.0^{f} (± 0.0)	10.0^{e} (± 1.71)	20.6^{d} (± 3.10)	0.0^{f} (± 0.0)
Mentha microphylla	71.0^{b} (± 12.78)	57.0^{a} (± 0.23)	90.5^{a} (± 8.26)	100.0^{a} (± 0.0)	100.0^{a} (± 0.0)	82.7^{a} (± 5.20)	100.0^{a} (± 0.0)	100.0^{a} (± 0.0)	100.0^{a} (± 0.0)	83.3^{a} (± 0.0)

Aa = *Alternaria alternata*, Bc = *Botrytis cinerea*, Bf = *Botrytis fabae*, Fc = *Fusarium culmorum*, Fo = *Fusarium oxysporum*, Hs = *Helminthosporium sp.*, Pd = *Penicillium digitatum*, Rso = *Rhizoctonia solani*, Rst = *Rhizopus stolonifer*, Sr = *Sclerotium rolfsii*.

Data are expressed as means ± S.D. from experiments with three replicates.

Means within a column sharing the same letter are not significantly different at the 0.05 probability level.

Table (2). Comparative *in vitro* antifungal activity of the extracted essential oils against plant pathogenic fungi

Oil	Fungi	EC_{50}[a] (mg/l)	95% Confidence limits (mg/L)		Slope[b] ± S.E.	Intercept[c] ± S.E.	(X^2)[d]
			Lower	Upper			
A. judaica	Aa	302.45	225.57	475.41	1.14 ± 0.26	-2.83 ± 0.62	0.07
	Fc	377.40	345.74	418.35	3.40 ± 0.46	-8.75 ± 1.18	0.69
	Hs	418.62	366.37	515.40	2.61 ± 0.81	-6.85 ± 2.10	0.01
	Pd	138.49	101.75	177.09	0.98 ± 0.15	-2.10 ± 0.35	4.45
	Rso	247.23	236.27	257.99	10.53 ± 0.95	-25.19 ± 2.30	0.45
	Rst	59.77	46.95	73.08	1.37 ± 0.16	-2.43 ± 0.31	0.96
A. santolina	Aa	461.72	431.44	509.70	5.41 ± 0.89	-14.42 ± 2.33	0.70
	Fc	335.8	281.8	402.5	1.82 ± 0.43	-4.59 ± 1.09	0.03
	Pd	335.68	283.65	399.04	1.88 ± 0.43	-4.76 ± 1.09	1.38
	Rst	448.54	423.37	483.85	6.26 ± 0.91	-16.61 ± 2.37	0.17
E. camaldulensis	Aa	348.47	329.50	365.19	7.91 ± 0.94	-20.12 ± 2.42	0.18
	Bf	430.55	375.50	518.42	2.23 ± 0.28	-5.88 ± 0.70	3.41
	Fc	394.71	294.39	705.59	0.89 ± 0.23	-2.31 ± 0.57	0.17
	Hs	481.76	429.26	625.01	3.12 ± 0.83	-8.38 ± 2.15	1.88
	Pd	85.50	58.90	115.75	0.78 ± 0.12	-1.51 ± 0.26	0.46
	Rso	384.35	361.80	407.08	6.12 ± 0.87	-15.82 ± 2.25	0.01
	Rst	386.63	353.91	430.38	3.39 ± 0.47	-8.76 ± 1.18	2.38
E. citriodora	Rso	129.68	107.42	165.67	1.76 ± 0.22	-3.72 ± 0.42	0.62
L. camara	Aa	287.30	198.66	460.82	0.95 ± 0.25	-2.34 ± 0.61	0.02
	Hs	409.67	334.58	628.91	1.41 ± 0.43	-3.69 ± 1.09	0.02
	Rst	372.53	302.79	531.48	1.60 ± 0.30	-4.12 ± 0.72	0.23
M. microphylla	Aa	280.45	224.50	325.38	1.94 ± 0.43	-4.75 ± 1.09	1.47
	Bc	474.63	404.62	644.11	2.07 ± 0.45	-5.54 ± 1.13	2.98
	Bf	298.83	246.17	396.78	1.52 ± 0.29	-3.75 ± 0.69	2.01
	Fc	205.40	188.55	218.85	7.42 ± 1.04	-17.15 ± 2.46	0.28
	Fo	263.59	231.36	292.61	3.24 ± 0.61	17.84 ± 1.51	1.25
	Hs	312.95	286.90	339.12	3.74 ± 0.46	-9.33 ± 1.17	3.73
	Pd	63.50	39.60	83.90	1.23 ± 0.22	-2.21 ± 0.42	0.23
	Rso	200.27	143.45	251.90	5.69 ± 0.47	-13.10 ± 1.11	4.82
	Rst	174.82	156.50	192.50	3.37 ± 0.33	-7.56 ± 0.77	0.67
	Sr	243.91	142.68	372.55	2.12 ± 0.25	-5.06 ± 0.61	9.21
Thiophanate methyl	Aa	81.17	63.60	115.41	1.47 ± 0.20	-2.81 ± 0.33	1.77
	Bc	8.52	7.22	9.88	2.41 ± 0.30	-2.25 ± 0.30	0.56
	Bf	10.79	9.09	12.84	2.09 ± 0.28	-2.16 ± 0.30	0.30
	Fc	10.16	7.58	13.76	1.31 ± 0.15	-1.32 ± 0.17	0.47
	Fo	21.86	18.63	25.47	2.34 ± 0.29	-3.14 ± 0.40	0.25
	Hs	80.44	64.63	108.97	1.66 ± 0.21	-3.17 ± 0.35	1.67
	Pd	10.57	8.62	12.94	1.77 ± 0.27	-1.81 ± 0.29	0.15
	Rso	19.04	17.10	21.08	4.05 ± 0.35	-5.17 ± 0.47	1.89
	Rst	>500					
	Sr	0.33	0.0	2.22	0.34 ± 0.13	0.16 ± 0.18	0.05

Aa = *Alternaria alternata*, Bc = *Botrytis cinerea*, Bf = *Botrytis fabae*, Fc = *Fusarium culmorum*, Fo = *Fusarium oxysporum*, Hs = *Helminthosporium sp.*, Pd = *Penicillium digitatum*, Rso = *Rhizoctonia solani*, Rst = *Rhizopus stolonifer*, Sr = *Sclerotium rolfsii*.

[a] Concentration causing 50% mycelial growth inhibition.

[b] Slope of concentration-inhibition regression line.

[c] Intercept of regression line.

[d] Chi square values.

Effect of the essential oils on control of fungal growth in inoculated strawberries: The effect of five isolated essential oils on the control of fungal growth in inoculated strawberries was examined. The isolated essential oils were highly effective in reducing gray mold and soft rot incidence in strawberry fruits caused by *B. cinerea* and *R. stolonifer,* respectively (Tables 3 and 4). All of the tested oils reduced disease incidence percentages caused by both fungi in a concentration dependent manner. The oil of *E. camaldulensis* showed the highest reduction of gray mold among the tested oils. This oil caused complete protection of fruit at concentrations of 1000 and 2000 mg/l after three days of treatment. The oils of *A. judaica* and *A. santolina* revealed a good control of gray mold while *M. microphylla* oil exhibit the weakest activity among the tested oils. The oil of *E. camaldulensis* was more active than thiabendazole, the reference fungicide, in the control of gray mold at all of the tested concentrations. In the case of soft rot caused by *R. stolonifer*, the tested oils showed a promising disease control after three days of treatments. The tested oils were more effective than thiabendazole in disease control at the tested concentrations. The oil of *E. camaldulensis* exhibited the highest reduction the soft rot incidence, followed by the oils of *A. santolina* and *A. judaica*, while *L. camara* and *M. microphylla* oils revealed the lowest disease reduction.

Table (3). *In vivo* antifungal activity of the extracted oils against *Botrytis cinerea*.

Conc (mg/l)	Disease incidence (%) (±S.D.)					
	Aj	As	Ec	Lc	Mm	TBZ
Control	85.2[a] (± 6.42)	85.2[a] (± 6.42)	85.2[a] (± 6.42)	85.2[a] (± 6.42)	85.2[a] (± 6.42)	85.2[a] (± 6.42)
250	38.9[b] (± 9.62)	61.1[b] (± 6.42)	22.2[b] (± 9.62)	48.1[b] (± 6.42)	85.2[a] (± 6.42)	27.7[b] (± 9.62)
500	16.7[c] (± 3.50)	22.2[c] (± 9.62)	5.6[c] (± 4.82)	37.0[b] (± 6.42)	70.4[b] (± 6.42)	16.7[c] (± 0.0)
1000	11.1[c] (± 9.62)	5.6[d] (± 4.82)	0.0[c] (± 0.0)	37.0[b] (± 6.42)	52.7[c] (± 6.05)	0.0[d] (± 0.0)
2000	5.5[c] (± 4.82)	0.0[d] (± 0.0)	0.0[c] (± 0.0)	22.2[c] (± 2.17)	21.8[d] (± 1.20)	0.0[d] (± 0.0)

Aj = *Artemisia judaica,* As = *Achillea santolina,* Ec = *Eucalyptus camaldulensis,* Lc = *Lantana camara*, Mm = *Mentha microphylla,* TBZ = Thiabendazole.
Data are expressed as means ± S.D. from experiments with four replicates.
Means within a column sharing the same letter are not significantly different at the 0.05 probability level.

Table (4). *In vivo* antifungal activity of the extracted oils against *Rhizopus stolonifer*.

Conc (mg/l)	Disease incidence (%) (±S.D.)					
	Aj	As	Ec	Lc	Mm	TBZ
Control	88.9[a] (± 0.0)	88.9[a] (± 0.0)	88.9[a] (± 0.0)	88.9[a] (± 0.0)	88.9[a] (± 0.0)	88.9[a] (± 0.0)
250	44.4[b] (± 9.62)	27.8 [bc] (± 1.92)	38.9[b] (± 9.62)	59.3[b] (± 6.42)	59.2[b] (± 6.45)	83.3[a] (± 6.67)
500	22.2[c] (± 5.09)	33.3[b] (± 0.0)	16.7[c] (± 0.0)	37.0.5[c] (± 6.42)	29.6[c] (± 6.4)	72.2[b] (± 9.62)
1000	5.6[d] (± 4.82)	22.2[c] (± 5.09)	5.6[d] (± 4.82)	25.9[d] (± 6.51)	25.9[c] (± 6.41)	55.6[c] (± 1.93)
2000	5.6[d] (± 4.82)	5.6[d] (± 4.82)	0.0[d] (± 0.0)	14.8[e] (± 6.42)	7.4[d] (± 6.41)	44.4[d] (± 5.09)

Aj = *Artemisia judaica,* As = *Achillea santolina,* Ec = *Eucalyptus camaldulensis,* Lc = *Lantana camara*, Mm = *Mentha microphylla,* TBZ = Thiabendazole.
Data are expressed as means ± S.D. from experiments with four replicates.
Means within a column sharing the same letter are not significantly different at the 0.05 probability level.

Chemical composition of the isolated essential oils: The chemical composition of the eight essential oils obtained by hydrodistillation and /or steam distillation was analyzed using GC–MS. The essential oil major constituents are given in Table (5). The major constituents of the essential oils were piperitone (49.09 %) and camphor (34.49 %) in *A. judaica*, 1,6-dimethyl,1,5-cyclooactadiene (60.52 %) and fragranol (10.52 %) in *A. santolina*, 1,8-cineole (45.47 %) and (-)-spathulenol (32.37 %) in *E. camaldulensis*, Citronellal (50.46 %), 1,8-Cineole (15.89 %) and L-Citronellol (10.54 %) in *E. citriodora*, 1,8-Cineole (55.71 %), α- Pinene (18.70 %) and *P*-Cymene (18.06 %) in *E. smithii*, *trans*-caryophyllene (42.63 %), α-humulene (13.94 %) and 1,8-cineole (13.34 %) in *L. camara*, piperitenone oxide (46.70 %) and piperitone oxide (28.0 %) in *M. microphylla*, and 4-terpineol (29.96 %) and β-terpinene (11.34 %) in *M. hortensis*. Some of major components were found in more than one plants such as (-)-spathulenol, 1,8-cineole, camphor and sabinene whereas others were specific to the plant species. The major constituents of the essential oils belong mainly to four groups: oxygenated monoterpenes (piperitone, camphor, fragranol, 1,8-cineole, piperitenone oxide, piperitone oxide, citronellal, L-citronellol and 4-terpineol), monoterpene hydrocarbons (1,6-

dimethyl,1,5-cyclooactadiene, *dl*-limonene, α-thujene, γ- terpinene, α-pinene, *P*-cymene and α-thujene), sesquiterpene hydrocarbons (*trans*-caryophyllene, α-humulene and γ-muurolene) and oxygenated sesquiterpenes ((-)-spathulenol).

Table (5). Major components of the isolated essential oils

Plant oil	Major components (%)
Mentha microphylla	Piperitenone oxide (46.70)
	Piperitone oxide (28.0)
	1,8-Cineole (13.34)
	Sabinene (3.50)
Lantana camara	*trans*-Caryophyllene (42.63)
	α-Humulene (13.94)
	1,8-Cineole (12.66)
	(-)-Caryophyllene (7.60)
Eucalyptus citriodora	Citronellal (50.46)
	1,8-Cineole (15.89)
	L-Citronellol (10.54)
	trans-Caryophyllene (5.24)
Eucalyptus smithii	1,8-Cineole (55.71)
	α- Pinene (18.70)
	P-Cymene (18.06)
	δ-3-Carene (5.47)
Eucalyptus camaldulensis	1,8-Cineole (45.47)
	(-)-Spathulenol (32.37)
	Bicyclogermacrene (11.20)
	Bicycloelemene (3.27)
Artemisia judaica	Piperitone (49.09)
	Camphor (34.49)
	Borneol (3.90)
Achillea santolina	Fragranol (10.52)
	β-Thujone (8.96)
	1,8-Cineole (8.64)
	Camphor (5.41)
Majorana hortensis	4-Terpineol (29.96)
	β-Terpinene (11.34)
	trans-Sabinene hydrate (10.81)
	α-Terpinene (6.77)

DISCUSSION

The results of the present study demonstrated that some of the isolated oils possessed a remarkable antifungal activity. *In vitro* experiments indicated that the oils of *M. microphylla*, *E. camaldulensis* and *A. judaica* were most effective in inhibition of mycelial growth of the tested fungi. Oils of *M. microphylla*, *E. camaldulensis*, *A. judaica*, *A. santolina* and *L. camara* were more effective than the reference fungicide, Thiophanate methyl, against *R. stolonifer*. The *In vitro* antifungal activity of the essential oils has been reported against plant pathogenic and postharvest fungi. It has been reported that some of the essential oils of medicinal plants inhibited the germination of spores and mycelial growth of plant pathogenic fungi (Fiori *et al.,* 2000; Bajpai *et al.,* 2007). In addition, the vapour phase of essential oils possessed antifungal activity against common postharvest fungal pathogens (Lee *et al.,* 2007; Szczerbanik *et al.,* 2007).

When tested for their potential to control postharvest diseases caused by *B. cinerea* and *R. stolonifer* on strawberries, the essential oils reduced gray mold and soft rot incidences in a dose dependant manner with oils of *E. camaldulensis*, *A. judaica* and *A. santolina* being the most effective. Few studies have been reported on the potential use of the essential oils in the control of postharvest disease caused by fungi (Reddy *et al.,* 1998; Feng and Zheng, 2007; Regnier *et al.,* 2008).

The major constituents of the isolated essential oils from *A. judaica*, *A. santolina*, *E. camaldulensis*, *E. citriodora*, *E. smithii*, *L. camara*, *M. microphylla*, and *M. hortensis* were similar to those previously reported on chemistry of these oils isolated from plants growing in Egypt or other countries (Bignell *et. al.,* 1998; Traboulsi *et al.,* 2002, 2005; Bader *et al.,* 2003; Tsiri *et al.,* 2003; Zrira *et. al.,* 2004; El-Ghorab *et al.,* 2004; Abdel-Hady *et al.,* 2005; Njoroge *et al.,* 2005; Su *et. al.,* 2006). However, the concentrations of the major compounds were significantly and/or slightly different. These changes in the essential oil compositions might arise from several environmental (climatical, seasonal, geographical) and genetic differences (Ravid *et al.,* 1992; Perry *et al.,* 1999).

Comparing the results obtained from both *in vitro* and *in vivo* experiments on *B. cinerea* and *R. stolonifer* revealed that the oils of *M. microphylla*, *E. camaldulensis* and *A. judaica* were the most effective in the *in vitro* assay against *B. cinerea*, while the oils of *E. camaldulensis*, *A. judaica* and *A.*

santolina were the most effective in control of gray mold on inoculated strawberries. On the other hand, the oils of *E. camaldulensis*, *A. judaica*, *A. santolina* and *M. microphylla* were the most effective against *R. stolonifer in vitro* and they were also the most effective in control of soft rot on inoculated strawberries.

The bioactivity of essential oils is mainly attributed to their monoterpenoidal constituents. The high antifungal activity of the oils of *M. microphylla*, *E. camaldulensis*, *A. judaica* and *A. santolina* may be associated to their high contents of monoterpenoids such as piperitenone oxide, piperitone oxide, piperitone, thujone, camphor and 1,8-Cineole. The antifungal activity of these oxygenated monoterpens has been reported (Jalsenjak *et al.,* 1987; Oumzil *et al.,* 2002; Cardenas-Ortega *et al.,* 2005; Kordali *et al.,* 2005; Romagnoli *et al.,* 2005; Saleh *et al.,* 2006; Terzi *et al.,* 2007; Abdelgaleil *et al.,* 2008).

The antifungal activity of essential oils may be attributed to the interference of oxygenated monoterpenes with certain enzymatic reactions evolved in the cell wall synthesis. Further, the mode of antifungal action of the essential oils could be dependent on two different mechanisms. Some oil components may irreversibly disrupt the cell membrane structure by cross linking reactions, causing a leakage of electrolytes and subsequent depletion of amino acids and sugars; while others may selectively be inserted into the lipid rich portion of the cell membrane, thereby disturbing membrane function (Zambonelli *et al.,* 1996, Inouye *et al.,* 2000).

In conclusion, this study demonstrates the antifungal activity of eight isolated essential oils against certain plant pathogenic fungi. As *in vitro* studies show the oils of *M. microphylla*, *E. camaldulensis* and *A. judaica* exhibited a strong antifungal activity. Further, these oils and *A. santolina* oil revealed a promising reduction of gray mold and soft rot incidences in strawberry fruits, caused by *B. cinerea* and *R. stolonifer*, respectively. As *M. microphylla* and *A. santolina* widely grow as wasteland weeds, and as *E. camaldulensis* is common tree in Egypt, their essential oils could become a renewable source for natural fungicides. However, further toxicological and biochemical studies are needed before implementation in fungi control programs.

REFERENCES

Abdelgaleil, S.A., Abbassy, M.A., Belal, A.S. and Abdel Rasoul, M.A.(2008). Bioactivity of two major constituents isolated from the essential oil of *Artemisia judaica* L. Bioresour. Technol., 99: 5947-5950.

Abdel-Hady, N.M., Abdel-Halim, A.S. and Al-Ghadban, A.M. (2005). Chemical composition and insecticidal activity of the volatile oils of leaves and flowers of *Lantana camara* L. cultivated in Egypt. J. Egyp. Soc. Parasitol., 35: 687-698.

Bader, A., flamini, G., Cioni, P.L. and Morelli, I. (2003). Essential oil composition of *Achillea santolina* L. and *Achillea biebersteinii* Afan. collected in Jordon. Flav. Frag. J., 18: 36-38.

Bajpai, V.K., Rahman, A. and Kang, S.C. (2007).Chemical composition and anti-fungal properties of the essential oil and crude extracts of *Metasequoia glyptostroboides* Miki ex Hu. Ind. Crops Prod., 26: 28–35.

Bignell, C.M., Dunlop, P.J. and Brophy, J. J. (1998). Volatile leaf oils of some south-western and southern Australian species of the genus *Eucalyptus* (series 1). Part XIX. Flav. Frag. J.,13: 2131 – 139.

Brent, K.J. and Hollomon, D.W. (1998). Fungicide resistance: the assessment of risk. FRAC, Global Crop Protection Federation, Brussels, Monograph No. 2, pp. 1–48.

Burt, S. (2004). Essential oils: their antibacterial properties and potential applications in foods- a review. Int. J. Food Microbiol., 94: 223-253.

Cárdenas-Ortega, N.C., Zavala-Sánchez, M.A., Aguirre-Rivera, J.R., Pérez-González, C. and Pérez-Gutiérrez, S. (2005). Chemical composition and antifungal activity of essential oil of *Chrysactinia mexicana* gray. J. Agric. Food Chem., 53: 4347-4349.

Cohort Software Inc. (1985). Costat User's Manual. Version 3. Cohort Tucson, Arizona, USA.

Cowan, M.M. (1999). Plant products as antimicrobial agents. Clin. Microbiol. Rev., 12: 564–582.

El-Ghorab, A.H., Mansour, A.F. and El-Massry, K.F. (2004). Effect of extraction methods on the chemical composition and antioxidant activity of Egyptian marjoram (*Marjorna hortensis* Moench). Flav. Frag. J., 19: 54-61.

Feng, W. and Zheng, X.(2007). Essential oils to control *Alternaria alternata in vitro* and *in vivo*. Food Control, 18: 1126–1130.

Finney, D.J. (1971). Probit Analysis, 3rd ed. Cambridge University Press, London, p. 318.

Fiori, A.C.G., Schwan-Estrada, K.R.F., Stangarlin, J.R., Vida, J.B., Scapim, C.A., Cruz, M.E.S. and Pascholati, S.F. (2000). Antifungal activity of leaf extracts and essential oils of some medicinal plants against *Didymella bryoniae*. J. Phytopathology, 148: 483-487.

Inouye, S., Tsuruoka, T., 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.

Jalsenjak, V., Peljnajk, S. and Kustrak, D.(1987). Microcapsules of sage oil, essential oils content and antimicrobial activity. Pharmazie, 42: 419–420.

Kordali, S., Cakir, A., Mavi, A., Kilic, H. and Yildirim, A. (2005). Screening of chemical composition and antifungal and antioxidant activities of the essential oils from three Turkish *Artemisia* species. J. Agric. Food Chem., 53:1408-1416.

Lee, S.O., Park, I.K., Choi, G.J., Lim, H.K., Jang, K.S., Choi, K.Y., Shin, S.C. and Kim, J.C. (2007). Fumigant activity of essential oils and components of *Illicium verum* and *Schizonepeta tenuifolia* against *Botrytis cinerea* and *Colletotrichum Gloeosporioides*. J. Microbiol. Biotechnol., 17: 1568–1572.

Njoroge, S.M., Koaze, H., Mwaniki, M., Minh Tu, N.T. and Sawamura, M. (2005). Essential oils of Kenyan *Citrus fruits*: volatile components of two varieties of mandrins (*Citrus reticulata*) and a tangelo (*C. paradise x C. tangerina*). Flav. Frag. J., 20: 74-79.

Oumzil, H., Ghoulami, S., Rhajaoui, M., Ilidrissi, A., Fkih-Tetouani, S., Faid, M. and Benjouad, A.(2002). Antibacterial and antifungal activity of essential oils of *Mentha suaveolens*. Phytother. Res., 16:727-31.

Pandey, D.K., Tripathi, N.N., Tripathi, R.D. and Dixit, S.N.Z. (1982). Fungitoxic and phytotoxic properties of essential oil of *Hyptis suaveolens*. PflKrankh PflSchutz, 89: 344-349.

Perry, N.B., Anderson, R.E., Brennan, N.J., Douglas, M.H., Heaney, A.J., McGrimpsey, J.A. and Smallfield, B.M. (1999). Essential oil from Dalmation sage (*Salvia officinalis* L.), variations among individuals, plant parts, seasons and sites. J. Agric. Food Chem., 47: 2048–2054.

Ravid, U., Putievsky, E., Katzir, I., Carmali, D., Eshel, A. and Schenk, H. B. (1992). The essential oil of *Artemisia judaica* L. chemotypes. Flav. Frag. J., 7: 69-72.

Reddy, M.V.B., Angers, P., Gosselin, A. and Arul, J. (1998). Characterization and use of essential oil from *Thymus vulgaris* against *Botrytis cinerea* and *Rhizopus stolonifer* in strawberry fruits. Phytochemistry, 47: 1515-1520.

Regnier, T., Plooy, W., Combrinck, S. and Botha, B. (2008). Fungitoxicity of *Lippia scaberrima* essential oil and selected terpenoid components on two mango postharvest spoilage pathogens. Postharvest Biol. Technol., 48: 254–258.

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.

Saleh, M.A., Belal, M.H. and El-Baroty, G. (2006). Fungicidal activity of *Artemisia herba alba* Asso (Asteraceae). J. Environ. Sci. Health B, 41: 237-244.

Su, Y.C., Ho, C. L., Wang, E. I. C. and Chang, S. T. (2006). Antifungal activities and chemical compositions of essential oils from leaves of four *Eucalyptus*. Taiwan J. For. Sci., 21:49-61.

Szczerbanik, M., Jobling, J., Morris, S. and Holford, P. (2007). Essential oil vapours control some common postharvest fungal pathogens. Australian J. Exper. Agric., 47: 103-109.

Tackholm, V. (1974). Student Flora of Egypt, 2nd Edition. Cairo University Press, Beirrut.

Terzi, V., Morcia, C., Faccioli, P., Valè, G., Tacconi, G. and Malnati, M.(2007). *In vitro* antifungal activity of the tea tree (*Melaleuca alternifolia*) essential oil and its major components against plant pathogens. Lett. Appl. Microbiol., 44: 613-618.

Traboulsi, A.F., El-Haj, S., Tueni, M., Taoubi, K., Nader, N.A. and Mrad. A. (2005). Repellency and toxicity of aromatic plant extracts against the mosquito *Culex pipiens molestus* (Diptera: Culicidae). Pest Manag. Sci., 61: 597-604.

Traboulsi, A.F., Taoubi, K., El-Haj, S., Bessiere, J.M. and Rammal, S. (2002). Insecticidal properties of essential plant oils against the mosquito *Culex pipiens molestus* (Diptera: Culicidae). Pest Manag. Sci., 58: 491-495.

Tsiri, D., Kretsi, O., Chinou, I.B. and Spyropoulos, C.G. (2003). Composition of fruit volatiles and annual changes in the volatiles of leaves of *Eucalyptus camaldulensis* Dehn. Growing on Greece. Flav. Frag. J., 18: 244-247.

Zambonelli, A., Zechini d'Aulerio, A., Bianchi, A. and Albasini, A. (1996). Effects of essential oil on phytopathogenic fungi. Phytopathology, 144: 491-494.

Zrira, S., Bessiere, J.M., Menut C., Elamrani A. and Benjilali B.(2004). Chemical composition of the essential oil of nine *Eucalyptus* species growing in Morocco. Flav. Frag. J., 19: 172 – 175.

النشاط الإبادى الفطرى ضد الفطريات الممرضة للنبات و مكافحة العفن الرمادى والطرى فى الفراولة بإستخدام الزيوت النباتية

سعاد محمدين أحمد و سمير عبد العظيم محمد عبد الجليل
قسم كيمياء المبيدات – كلية الزراعة (الشاطبى)- جامعة الاسكندرية – مصر

تم الحصول على الزيوت الطيارة لثمانية نباتات هى الشيح و البعثران و وثلاثة أنواع من الكافور واللنتانا و البردقوش والفلية بواسطة التقطير المائى والتقطير بالبخار. تم تقدير النشاط الإبادى الفطري للزيوت المعزولة ضد عشرة فطريات ممرضة للنبات وهى *Alternaria alternata* و *Botrytis cinerea* و *B. fabae* و *Fusarium culmorium* و *F. oxysporum* و *Helminthosporuim sp.* و *Penecillium digitatum* و *Rhizoctonia solani* و *Rhizopus stolonifer* و *Sclerotium rolfsii* . خمسة من الزيوت المعزولة تم تقييمها لمكافحة عفن الثمار فى الفراولة والمتسببة عن فطرى *Botrytis cinerea* و *Rhizopus stolonifer*. فى إختبارات معملية *In vitro* أحدث زيت الفلية تثبيطاً كاملاً لفطريات *F. culmorium* و *F. oxysporum* و *P. digitatum* و *R. solani* و *R. stolonifer* على تركيز 500 مجم/لتر. بينما سبب زيتى الشيح و كافور *E. citriodora* تثبيطاً كاملاً لفطر *R. solani* عند نفس التركيز. أظهرت دراسات السمية الفطرية المقارنة أن زيت الفلية هو أكثر الزيوت المختبرة فاعلية يلية زيت الكافور البلدى و الشيح. كما أظهرت الزيوت حفضا قويا فى مستوى الإصابة عند الإختبار لمكافحة العفن الرمادى والعفن الطرى فى الفروالة. زيوت الكافور والشيح والبعثران كانت أكثر فاعلية فى مكافحة المرضيين. لربط العلاقة بين النشاط الإبادى الفطرى و التركيب الكيماوى للزيوت تم تحليل الزيوت بواسطة جهاز الكروماتوجرافى الغازى- مطياف الكتلة. المركبات السائدة فى الزيوت كانت مكونة من أربع مجاميع كيماوية رئيسية هى oxygenated monoterpenes و monoterpene hydrocarbons و sesquiterpene hydrocarbons و oxygenated sesquiterpenes. التأثير الإبادى الفطرى الجيد لزيوت الكافور والشيح والبعثران الذى أظهرته هذه الدراسة يعطى دلالة على إمكانية إستخدام هذه الزيوت كوسائل بديلة لمكافحة الأمراض الفطرية بعد الحصاد.