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Full Length Research Paper

Evaluation of antibacterial properties and biochemical effects of monoterpenes on plant pathogenic bacteria

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The antibacterial activity of twelve monoterpenes, namely camphene, (*R*)-camphor, (*R*)-carvone, 1,8-cineole, cuminaldehyde, (*S*)-fenchone, geraniol, (*S*)-limonene, (*R*)-linalool, (*1R,2S,5R*)-menthol, myrcene and thymol was tested against two plant pathogenic bacteria *Agrobacterium tumefaciens* and *Erwinia carotovora* var. *carotovora* using agar dilution method. For a better understanding of monoterpenes mechanisms of action, the inhibitory effect of three monoterpenes (*R*)-linalool, myrcene and thymol was assessed on dehydrogenases and polyglacturonase activities. Among the tested monoterpenes, thymol, (*S*)-limonene and myrcene were the most potent antibacterial compounds against *A. tumefaciens* with minimum inhibitory concentration (MIC) of 1000 mg/L. Thymol was also the most effective compounds against *E. carotovora* var. *carotovora*, while camphene, cunimaldhyde and 1,8-cineole were the less effective compounds against both bacteria. In biochemical studies, myrcene caused the highest inhibitory effect on dehydrogenases activity of the two tested bacteria, followed by thymol. However, thymol showed the highest inhibitory effect on polygalacturonase activity of both tested bacteria, followed by (*R*)-linalool. In general, there was a positive correlation between the antibacterial activity of monoterpenes and their inhibitory effects on both enzymes. This is the first report for the determination of MIC and enzymes inhibitory effects of tested monoterpenes on plant pathogenic bacteria.

Key words: Monoterpenes, antibacterial activity, plant pathogenic bacteria, dehydrogenases, polygalacturonase.

INTRODUCTION

Monoterpenes are a class of natural products containing ten carbons, found in many different higher-order plants and are the main constituents in the majority of plant essential oils. Two thousands naturally occurring monoterpenes are known. These compounds give plants their unique odoriferous properties. They are derived from the coupling of two isoprenoid units, which are made from isopentylpyrophosphate, a precursor in the biosynthesis of cholesterol (Windholz et al., 1983). Monoterpenes can be classified into two major groups: monoterpene hydrocarbons and oxygenated monoterpenes. The latter group includes alcohols, aldehydes, ketones, ethers and acids (Templeton, 1969). These compounds are usually fragrant oils or low melting solids, which are often found in perfumes and other cosmetics, and are commonly used as food additives and therapeutic drugs (Tsao and Coats, 1995). The natural pesticidal properties of some monoterpenes make them useful as potential alternative pest control agents as well as good lead compounds for the development of safe, effective, and fully biodegradable pest control agents. Monoterpenes have been shown to possess remarkable pesticidal activities, including insecticidal (Isman, 2000; Grodnitzky and Coats, 2002), herbicidal (Duke et al., 2000; Singh et al., 2002), fungicidal (Wuryatmo et al.,

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2003; Cärdenas-Ortega et al., 2005) and bactericidal (Cristani et al., 2007; Cantore et al., 2009) properties.

Agrobacterium tumefaciens, a soil borne bacterium, causes crown gall which is a world wide disease of a wide range of dicotyledonous plants, especially members of the rose family such as apple, pear, peach, cherry, almond, raspberry and roses. It is estimated that *A. tumefaciens* causes crown gall disease in over 600 species of trees (Wang et al., 2000). This disease cause great economic loss in fruit plants (Wang et al., 1991; Abussaoud and Al-Momani, 1992). *Erwinia carotovora* subsp. *carotovora* is a soil borne facultative anaerobic pathogen. It infects and causes soft rot, blackleg or stem rot in many economically important crops, including vegetables, flowers and fruits (Pérombelon and Kelman, 1980). This bacterium invade crops in the fields or in storage and causes plant tissues to become soft and watery which then turn slimy and foul-smelling, eventually resulting in plant death (Pérombelon and Kelman, 1980; Wright, 1998).

Control of phytopathogenic bacteria encountered several difficulties, such as the lack of effective bactericides (Montesinos et al., 2000), the development of resistance for many of these pathogens, and the wide host range of some these bacteria (Loper et al., 1991; Sundin and Bender, 1993). For many years, copper derivatives and antibiotics are used for the control of these pathogens. However, the intensive use of these chemicals caused serious environmental contaminations and emergence of resistance which limited the value of antibiotics in crop protection. Therefore, there is an urgent need for new chemicals and/or products for control phytopathogenic bacteria. Thus, there has been a growing interest on the research of the possible use of plant secondary metabolites for pest and disease control in agriculture (Costa et al., 2000). Essential oils and their major constituents, monoterpenes are among the most promising classes of natural products that can be used as safer pest and disease control agents.

Although several studies have been reported on the antibacterial activity of monoterpenes against human and animal pathogens, food poisoning and spoilage bacteria (Naigre et al., 1996; Dorman and Deans, 2000; Cantrell et al., 2001; Inouye et al., 2001; Trombetta et al., 2005; Si et al., 2006; Cristani et al., 2007; Kotan et al., 2007; Zarrini et al., 2010; Garcìa-Garcìa et al., 2011), there were a few studies in the literature on the activity of monoterpenes against plant pathogenic bacteria. For examples, the *in vitro* antimicrobial activity of geraniol towards 7 strains of *Erwinia amylovora*, the causal agent of 'fire blight' of rosaceous plants, was assessed in tube cultures by Scortichini and Rossi (2008) and Sato et al. (2007). El-Zemity et al. (2008) evaluated antibacterial effects of 13 monoterpenes against *A. tumefaciens* and *E. carotovora* using inhibition zone method. Eugenol had a potential to disinfect bean seeds from *Xanthomonas campestris* pv. *phaseoli* var. *fuscans* (Cantore et al., 2009).

In the present study, the antibacterial activity of 12 monoterpenes camphene, (*R*)-camphor, (*R*)-carvone, 1,8-cineole, cuminaldehyde, (*S*)-fenchone, geraniol, (*S*)-limonene, (*R*)-linalool, (*1R,2S,5R*)-menthol, myrcene and thymol was evaluated against two plant pathogenic bacteria *A. tumefaciens* and *E. carotovora*. The inhibitory effect of these monoterpenes was examined on the activity of two exocellular enzymes dehydrogenases and polygalacturonase to explore their possible mode of action.

MATERIALS AND METHODS

Chemicals

Twelve monoterpenes, camphene (95%), (*R*)-camphor (98%), (*R*)-carvone (98%), 1-8-cineole (99%), cuminaldehyde (98%), (*S*)-fenchone (98%), geraniol (98%), (*S*)-limonene (96%), (*R*)-linalool (95%), (*1R,2S,5R*)-menthol (98%), myrcene (90%) and thymol (98%) were purchased from Sigma–Aldrich Chemical Co., Steinheim, Germany. Chemical structures of these monoterpenes are shown in Figure 1.

Test bacteria

Bacteria of crown gall disease *A. tumefaciens* (E. F. Smith & Town.) (Family: Rhizobiaceae; Class: Alpha Proteobacteria) and soft mold disease *E. carotovora* var. *carotovora* (Family: Enterobacteriaceae; Class: Gamma Proteobacteria) were provided by Microbiology Laboratory, Department of Plant Pathology, Faculty of Agriculture, Alexandria University, Egypt. The bacterial strains were maintained on nutrient agar medium (NA: peptone 10, meat extract 5, sodium chloride 2.5 and agar 10 g litre^{-1} in distilled water) at 37°C).

Minimum inhibitory concentration (MIC) assay

Agar dilution method assay was used as recommended by European Society of Clinical Microbiology and Infectious Diseases (ESCMID, 2000) for determination of MIC. The tested monoterpenes were dissolved in acetone. Appropriate volumes of the stock solutions were added to molten NA to obtain a range of concentrations (10 to 10000 mg/L) before pouring to Petri dishes. After solidifications, 6 µL of bacterial cultures (approximately 10^8 CFU/mL) was spotted (three spots per each plate) using 2 µL standard loop on the surface of agar. The inoculum spots were allowed to dry before inverting the plates for incubation at 37°C for 24 h. The MIC was determined as lowest concentration of monoterpenes showing no visible bacterial growth in the agar plates.

Dehydrogenases activity assay

The bacterial strains (*A. tumefaciens* and *E. carotovora*) were grown in nutrient broth (NB) medium supplemented with 0.1% pectin as a sole source carbon for 48 h at 30±2°C. Me thylene blue method recommended by Schoenhard (1962) was used for the determination of dehydrogenases activity. The reaction mixture containing 2.0 ml phosphate buffer (pH 7), 1.0 ml methylene blue (0.0001%), 2.0 ml bacterial suspension (A_{420} = 0.71), 1.0 ml glucose (2×10^{-2} M) and 4.0 ml tested monoterpene solution was

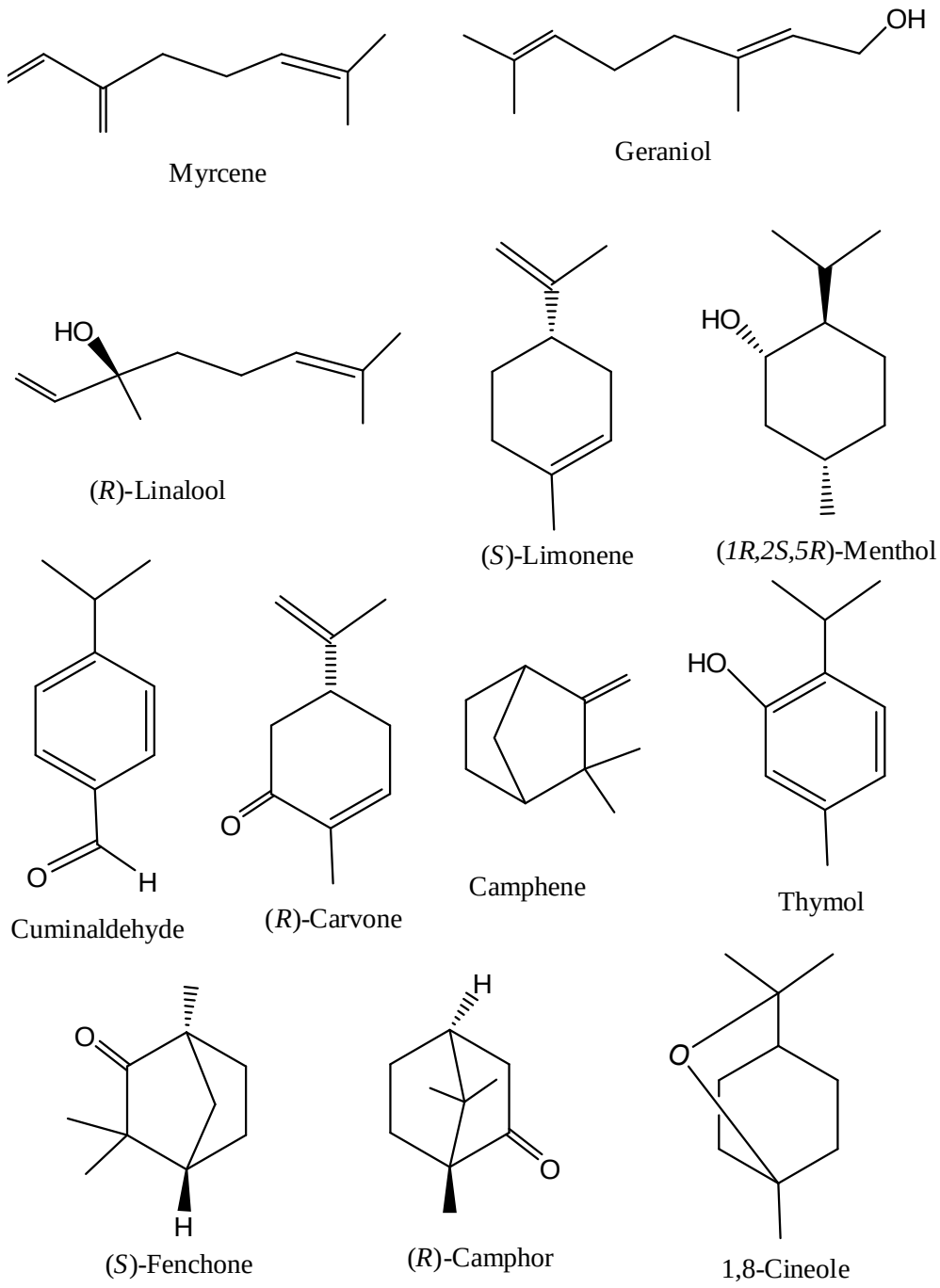

Figure 1. The chemical structure of monoterpenes.

prepared in a test tube. Thereafter, 2 ml of mineral oil was added to each test tube to isolate the reaction mixture from the aerial oxygen. Three replicates of each concentration and control were carried out. The treatments were kept at 28°C. The monoterpenes (myrcene, (*R*)-linalool and thymol) were tested at final concentrations of 10, 50, 100, 500 and 1000 mg/L. The rate of the 90% anaerobic reducing of methylene blue was taken as criterion for the activity of the dehydrogenase. Percentage of inhibition (I%) was calculated as follows:

$$I\ (\%) = ((T - C)/ T_{max} - C) \times 100,$$

Where: T = time, (min), required for the 90% reduction of methylene blue in the treatments, T_{max} = the maximum time for the 90% reduction in MB reached by the strongest enzyme inhibition, and it was 615 minutes, C = time of the 90% reduction in MB in control treatment. The concentration of monoterpene caused 50% of enzyme inhibition (IC_{50}) was calculated using Probit analysis (Finney, 1971).

Table 1. Minimum inhibitory concentration (MIC) of monoterpenes on *A. tumefaciens* and *E. carotovora*.

Compound	Minimum inhibitory concentration (MIC, mg/L)	
	A. tumefaciens	***E. carotovora***
Camphene	5000	>5000
Thymol	1000	2000
Cunimaldhyde	5000	>5000
(*R*)-Carvone	2500	5000
(*R*)-Camphor	2500	4000
(*1R,2S,5R*)-Menthol	2500	4000
(*S*)-Fenchone	2500	5000
Geraniol	1500	4000
(*R*)-Linalool	2500	4000
(*S*)-Limonene	1000	5000
Myrcene	1000	3000
1,8-Cineole	4000	>5000

Polygalacturonase activity assay

Polygalacturonase reaction mixture [0.5% polygalacturonic acid in 0.05 M sodium acetate buffer, pH 5.5, 0.1 M NaCl, 0.35 ml citrate buffer (5.5 pH), 4.0 ml polygalacturonic acid (0.5%), 0.15 ml NaCl (0.1 M) and 0.5 ml bacterial suspension (Nasuno and Starr, 1966)] was incubated with different concentrations (10, 50, 100, 500 and 1000 mg/L) of myrcene, (*R*)-linalool and thymol for 2 h. The reaction mixture was centrifuged at 12000 rpm for 10 min. Then, 2 ml of the supernatant was mixed with an equal volume of thiobarbituric acid (TBA) reagent (Ayers et al., 1966) and heated at 100℃ for 30 min. The activity was determined as an increase in the absorbance at 550 nm (OD_{550}). Each treatment was replicated 3 times. Zero time reaction mixture containing active enzyme was used as control. Percentage of inhibition (I%) of polyglacturonase activity was calculated from the equation as follows:

$$I\ \% = ((A\ control - A\ treatment)/A\ control) \times 100$$

Where A is the absorbance.

RESULTS

Antibacterial effect of monoterpenes

Twelve monoterpenes with different chemical classes were tested for their antibacterial effect against two plant pathogenic bacteria *A. tumefaciens* and *E. carotovora*. The results showed that the tested monoterpenes possessed variable degrees of antibacterial activity (Table 1). In general, *A. tumefaciens* was more sensitive than *E. carotovora* to all of the tested monoterpenes. Thymol, (*S*)-limonene and myrcene were the most potent antibacterial compounds against *A. tumefaciens* with MIC value of 1000 mg/L. In contrast, camphene and cunimaldhyde were the least effective compounds. On the other hand, thymol (MIC = 2000 mg/L) showed the highest antibacterial activity against *E. carotovora*, followed by myrcene, while camphene, cunimaldhyde and 1-8 cineole had the lowest antibacterial activity since MIC values of these compounds were greater than 5000 mg/L.

Effect of monoterpenes on dehydrogenases activity

The inhibitory effect of the most antibacterial effective monoterpemes thymol, (*R*) linalool and myrcene was evaluated on dehydrogenases activity. The results showed that myrcene had the strongest inhibitory effect on the enzymes of both tested bacteria bacteria *A. tumefaciens* and *E. carotovora*, followed by thymol, while (*R*) linalool revealed the lowest inhibitory effect. The IC_{50} values of myrcene were 21.7 and 47.17 mg/L on the enzymes of *A. tumefaciens* and *E. carotovora*, respectively. The inhibitory effect of monoterpenes on dehydrogenases of *A. tumefaciens* was higher than that on dehydrogenases of *E. carotovora* in Table 2.

Effect of monoterpenes on polyglacturonase activity

Table 3 shows the inhibitory effect of the 3 monoterpenes thymol, (*R*) linalool and myrcene on the polyglacturonase activity of *A. tumefaciens* and *E. carotovora*. The tested monoterpenes caused potent inhibitory effect on the polyglacturonase activity of *A. tumefaciens* with thymol (IC_{50} = 14.46 mg/L) being the most effective, followed by (*R*) linalool (IC_{50} = 26.88 mg/L) and myrcene (IC_{50} = 38.83 mg/L). Similarly, thymol caused the highest inhibitory effect on polyglacturonase activity of *E. carotovora,* followed by (*R*) linalool and myrcene. Polyglacturonase of *A. tumefaciens* was more sensitive to inhibition by the tested monoterpenes than polyglacturonase of *E. carotovora*.

Table 2. Inhibitory effect of monoterpenes on dehydrogenases activity of *A. tumefaciens* and *E. carotovora*.

Compound	*A. tumefaciens*			*E. carotovora*		
	IC_{50}[a] (mg/L)	95% Confidence limits	Slope ± SE[b]	IC_{50} (mg/L)	95% Confidence limits	Slope ± SE
Thymol	50.49	42.74 - 59.24	2.32 ± 0.21	149.8	83.3 - 262.1	1.95 ± 0.14
(*R*)-Linalool	244.3	196.6 - 307.6	1.38 ± 0.11	351.2	-	0.78 ± 0.10
Myrcene	21.70	13.64 - 31.04	0.94 ± 0.10	47.17	31.17 - 67.88	0.79 ± 0.10

[a], The concentration causing 50% enzyme inhibition; [b], slope of the concentration-inhibition regression line.

Table 3. Inhibitory effect of monoterpenes on polyglacturonase activity of *A. tumefaciens* and *E. carotovora*.

Compound	*A. tumefaciens*			*E. carotovora*		
	IC_{50}[a] (mg/L)	95% Confidence limits	Slope ± SE[b]	IC_{50} (mg/L)	95% Confidence limits	Slope ± SE
Thymol	14.46	9.70 - 26.76	0.81 ± 0.09	36.55	40.88 - 75.34	0.99 ± 0.09
(*R*)-Linalool	26.88	16.56 - 39.06	0.84 ± 0.09	73.97	53.84 - 98.97	0.94 ± 0.09
Myrcene	38.83	5.82 - 97.97	0.67 ± 0.09	100.8	73.34 - 137.0	0.89 ± 0.09

[a], The concentration causing 50% enzyme inhibition; [b], slope of the concentration-inhibition regression line.

DISCUSSION

The present study shows that the 12 monoterpenes exhibited pronounced antibacterial inhibitory effect against the phytopathogenic bacteria *A. tumefaciens* and *E. carotovora*. To the best of our knowledge, this is the first report on the determination of MIC of these monoterpenes on the both tested bacteria. El-Zemity et al. (2008) evaluated some of the tested compounds such as (*R*)-camphor, (*R*)-carvone, 1,8-cineole, geraniol, (*1R,2S,5R*)-menthol and thymol against the tested bacteria using inhibition zone method and concluded that thymol was the most effective compound. Similarly, our results revealed that thymol had the highest antibacterial activity. It has been also reported that thymol was the most potent monoterpenes against plant pathogenic fungi (Muller-Riebau et al., 1995; Tsao and Zhou, 2000; Sokovic et al., 2002). The results also revealed that all of the tested compounds had stronger antibacterial activity against *A. tumefaciens* than *E. carotovora*. This indicated that the activity of monoterpenes varied with the species under the study.

It is well known that monoterpenes caused their antimicrobial inhibitory effects through the interaction with membrane structure and function. This is in fact due to their lipophilic and solubility properties. These interactions include membrane expansion, increase membrane fluidity and permeability, disturbance of membrane-embedded proteins, inhibition of respiration and alteration of ion transport processes (Uribe et al., 1983; Sikkema et al., 1994 and 1995; Cox et al., 2000; Prashar et al., 2003). In the present study, 3 of tested monoterpenes thymol, (*R*) linalool and myrcene showed strong inhibitory effects on the enzymes dehydrogenases and polyglacturonase of *A. tumefaciens* and *E. carotovora*. There was a positive correlation between the antibacterial activity and the polyglacturonase inhibition of both bacteria. However, the correlation was not clear in the case of dehydrogenases. The inhibition of both enzymes was concentration dependent. These results indicated that besides their previous effects on cell membrane, monoterpenes may cause their antibacterial activity through the inhibition of these enzymes.

Conclusion

We have evaluated the antibacterial activity of 12 monoterpenes with diverse chemical structures against the two plant pathogenic bacteria A. *tumefaciens* and *E. carotovora in vitro*. Among all of the tested compounds, thymol and myrcene were the most potent antibacterial activity. In addition, 3 monoterpenes thymol, (*R*) linalool and myrcene showed inhibitory activity on dehydrogenases and polyglacturonase. These results provided insight into the important of monoterpenes as possible control agents for plant pathogenic bacteria as well as contributing to a better understanding of their mechanisms of action.

REFERENCES

Abussaoud MJI, Al-Momani F (1992). Microbial survey of the genus *Agrobacterium* in grapevine nurseries in Jordan. Arab Gulf J. Scient. Res., 10: 121-131.

Ayers WA, Papavizacs G, Djema F (1966). Polygalacturonate trans-eliminase and polygalacturonase production by *Rhizcctonia solani.* Phytopathol., 56: 1006-1011.

Cantore PL, Shanmugaiah V, Iacobellis NS (2009). Antibacterial activity

of essential oil components and their potential use in seed disinfection. J. Agric. Food Chem., 57: 9454-9461.
Cantrell CL, Franzblau SG, Fischer NH (2001). Antimycobacterial plant terpenoids. Planta Med., 67: 685-694.
Cärdenas-Ortega NC, Zavala-Sänchez MA, Aguirre-Rivera JR, Peärez-Gonzälez C, Peärez-Gutienärrez S (2005). Chemical composition and antifungal activity of essential oil of *Chrysactinia mexicana* Gray. J. Agric. Food Chem., 53: 4347-4349.
Costa TR, Fernandes FLF, Santos SC, Oliveria CMA, Liao LM, Ferri PH, Paulo JR, Ferreira HD, Sales BHN, Silva MRR (2000). Antifungal activity of volatile constituents of *Eugenia dysenterica* leaf oil. J. Ethnopharmcol., 72: 111-117.
Cox SD, Mann, Markham JL , Bell HC, Gustafson JE, Warmington JR, Wyllie SG (2000). The mode of antimicrobial action of the essential oil of *Melaleuca alternifolia* (tea tree oil). J. Appl. Microbiol., 88: 170-175.
Cristani M, D'arrigo M, Mandalari G, Castelli F, Sarpietro MG, Micieli D, Venuti V, Bisignano G, Saija A, Trombetta D (2007). Interaction of four monoterpenes contained in essential oils with model membranes: Implications for their antibacterial activity. J. Agric. Food Chem., 55: 6300-6308.
Dorman HJD, Deans SG (2000). Antimicrobial agents from plants: antibacterial activity of plant volatile oils. J. Appl. Microbiol., 88: 308-316.
Duke SO, Romagni JG, Dayan FE (2000). Natural products as sources for new mechanisms of herbicidal action. Crop Protect., 19: 583-589.
El-Zemity SR, Radwan MA, Mohamed SA, Sherby SM (2008). Antibacterial screening of some essential oils, monoterpenoids and novel n-methyl carbamates based on monoterpenoids against *Agrobacterium tumefaciens* and *Erwinia carotovora*. Arch. Phytopathol. Plant Protect., 41: 451-461.
European Society of Clinical Microbiology and Infectious Diseases (ESCMID) (2000). Determination of minimum inhibitory concentrations (MICs) of antibacterial agents by agar dilution. Clin. Microbiol Infect., 6: 509-515.
Finney DJ (1971). Probit Analysis, 3rd ed. Cambridge University Press, London, p. 318.
García-García R, López-Malo A, Palou E (2011). Bactericidal action of binary and ternary mixtures of carvacrol, thymol, and eugenol against Listeria innocua. J. Food Sci., 76: 95-100.
Grodnitzky JA, Coats JR (2002). QSAR evaluation of monoterpenoids insecticidal activity. J. Agric. Food Chem., 50: 4576-4580.
Inouye S, Takizawa T, Yamaguchi H (2001). Antibacterial activity of essential oils and their major constituents against respiratory tract pathogens by gaseous contact. J. Antimicrob. Chemother., 47: 565-573.
Isman MB (2000). Plant essential oils for pest and disease management. Crop Protect., 19: 603-608.
Kotan R, Kordali S, Cakir A (2007). Screening of antibacterial activities of twenty-one oxygenated monoterpenes Z. Naturforsch., 62c: 507-513.
Loper JE, Henkels MD, Roberts RG, Grove GG, Willet MJ, Smith TJ (1991). Evaluation of streptomycin, oxytetracycline and copper resistance of *Erwinia amylovora* isolated from pear orchards in Washington state. Plant Dis., 75: 287-290.
Montesinos E, Melgarejo P, Cambra MA, Pinochet J (ed.) (2000). Enfermedades de los frutales de pepita y hueso. Ediciones Mundi Prensa, Barcelona, Spain.
Muller-Riebau F, Berger B, Yegen O (1995). Chemical composition and fungitoxic properties to phytopathogenic fungi of essential oils of selected aromatic plants growing wild in Turkey. J. Agric. Food Chem., 43: 2262-2266.
Naigre R, Kalck P, Roques C, Roux I, Michel G (1996). Comparison of Antimicrobial Properties of Monoterpenes and their Carbonylated Products Planta Med., 62: 275-277.
Nasuno S, Starr MP (1966). Polygalacturonase of *Erwinia carotovora.* J. Biol. Chem., 241: 5298-5306.
Pérombelon MCM, Kelman A (1980). Ecology of the soft rot Erwinias. Ann. Rev. Phytopathol., 18: 361-387.
Prashar A, Hili P, Veness RG, Evans CS (2003). Antimicrobial action of palmarosa oil (Cymbopogon martinii) on *Saccharomyces cerevisiae*. Phytochemistry, 63: 569-575.
Sato K, Krist S, Buchbauer G (2007). Antimicrobial effect of vapours of geraniol, (R)-(−)-linalool, terpineol, γ-terpinene and 1, 8-cineole on airbone microbes using an air washer. Flav. Fragr. J., 22: 435-437.
Schoenhard DE (1962). Basic Concepts and Experiments in Microbiology. Burgess Publishing Co. Minnesota, p. 246.
Scortichini M, Rossi MP (2008). *In vitro* susceptibility of *Erwinia amylovora* (Burrill) Winslow et al. to geraniol and citronellol. J. Appl. Microbiol., 71: 113-118.
Si W, Gong J, Tsao R, Zhou T, Yu H, Poppe C, Johnson R, Du Z (2006). Antimicrobial activity of essential oils and structurally related synthetic food additives towards selected pathogenic and beneficial gut bacteria. J. Appl. Microbiol., 100: 296-305.
Sikkema J, de Bont JA, Poolman B (1994). Interactions of cyclic hydrocarbons with biological membranes. J. Biol. Chem., 269: 8022-8028.
Sikkema J, de Bont JAM, Poolman B (1995). Mechanisms of membrane toxicity of hydrocarbons. Microbiol. Rev., 59: 201-222.
Singh HP, Batish DR, Kohli RK (2002). Allelopathic effect of two volatile monoterpenes against bill goat weed (*Ageratum conyzoides* L.). Crop Protect., 21: 347-350.
Sokovic M, Tzakou O, Pitarakoli D, Couladis M (2002). Antifungal activities of selected aromatic plants growing wild in Greece. Nahrung/Food, 46: 317-320.
Sundin GW, Bender CL (1993). Ecological and genetic analysis of copper and streptomycin resistance in *Pseudomonas syringae* pv. *syringae*. Appl. Environ. Microbiol., 59: 1018-1024.
Templeton W (1969). An introduction of chemistry of terpenoids and steroids. Butterworths, London.
Trombetta D, Castelli F, Sarpietro MG, Venuti V, Cristani M, Daniele C, Saija A, Mazzanti G, Bisignano G (2005). Mechanisms of Antibacterial Action of Three Monoterpenes. Antimicrob. Agents Chemother., 49: 2474-2478.
Tsao R, Coats JR (1995). Starting from nature to make better insecticides. Chemtech., 25: 23-38.
Tsao R, Zhou T (2000). Antifungal activity of monoterpenoids against postharvest pathogens *Botrytis cinerea* and *Monilinia fructicola*. J. Essential Oil Res., 12: 113-121.
Uribe S, Ramirez J, Pena A (1985). Effects of beta-pinene on yeast membrane functions. J. Bacteriol., 161: 1195-1200.
Wang H, Wang H, Wang J, Tzibun N (2000). The pathogen of crown gall disease on flowring cherry and its sensitivity to strain K1026. Eur. J. Plant Pathol., 106: 475-479.
Wang HM, Liang YJ, Yun T Di YB (1991). Biological control of crown gall with introduced K84 in China. I. Investigation and identification of *Agrobacterium tumefaciens*. Acta. Agri. Univ. Pekin., 17: 91-94.
Windholz M, Budavari S, Blumetti RF, Otterbein ES (1983). The Merck index. Merck, Rahway, NJ.
Wright PJ (1998). A soft rot of calla (*Zantedeschia spp.*) caused by *Erwinia carotovora* subspecies *carotovora*. New Zeal. J. Crop. Hort., 26: 331-334.
Wuryatmo E, Klieber A, Scott E (2003). Inhibition of citrus post-harvest pathogens by vapor of citral and related compounds in culture. J. Agric. Food Chem., 51: 2637-2640.
Zarrini G, Delgosha ZB, Moghaddam KM, Shahverdi AR (2010). Post-antibacterial effect of thymol. Pharm. Biol., 48: 633-636.