*Phytopathol. Mediterr.* (2003) 42, 251–256

# Antifungal activity of essential oils from several medicinal plants against four postharvest citrus pathogens

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**Summary.** Antifungal activity of 25 essential oils, distilled from Moroccan medicinal plants, against *Penicillium digitatum, Phytophthora citrophthora, Geotrichum citri-aurantii* and *Botrytis cinerea* is reported. Essential oil from *Chrysanthemum viscidehirtum* at a concentration of 150 ppm (v:v) strongly inhibited *in vitro* growth of all four fungi. The other 24 oils reduced fungal development less than 69% at a concentration of 250 ppm. *C. viscidehirtum* essential oil was further tested on citrus fruits (*Citrus reticulata* cv. Nules) inoculated with *P. digitatum*, *G. citri-aurantii* and *P. citrophthora* ($10^5$ conidia $ml^{-1}$). The antifungal activity of this oil was weak at 250 ppm, but at 2000 ppm the percentage of decayed fruits was very low. The inhibition data were compared to treatments with the synthetic fungicides procymidone, thiabendazole (TBZ), guazatine and propamocarbe HCL at 1000 ppm. GC-Mass spectrum analysis indicated that *C. viscidehirtum* essential oil contains ß-farnesene, limonene and many oxygenated sesquiterpenes.

**Key words:** fungitoxic activity, *Chrysanthemum viscidehirtum*, *Citrus* spp.

## Introduction

Concern among experts and the public about residues of synthetic fungicides ending up in foods and in the environment, as well as about the development of fungicide resistance in pathogens are the main driving forces behind a search for new chemical compounds with an original mode of action. The use of low-toxicity fungicides to control postharvest pathogens without danger to human health is extremely important. Several studies on the use of essential oils as fungicides have been published (Wilson *et al*., 1987; Shimoni *et al*., 1993; Arras *et al*., 1995; Carta *et al*., 1996; Cutler *et al*., 1996; Bhaskara *et al*., 1998; Arras and Usai, 2001).

In an attempt to find new applications for Moroccan aromatic plants used locally as remedies in traditional medicine, we started a programme to study the antifungal and pharmacological properties of the volatile fraction of these plants in the hope of finding new natural biologically-active compounds (El Mahi *et al*., 1998; Khalouki *et al*., 2000; Hmamouchi *et al*., 2001; Lahlou *et al*., 2001a, b). In this study, several essential oils from Moroccan medicinal plants were evaluated as agents to control four pathogenic fungi.

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## Materials and methods

### Plant collection and essential oil extraction

A number of aromatic plants were collected from different regions in Morocco. They were taxonomically identified at the National Scientific Institute of Rabat (Department of Plant Biology, Botany Laboratory). A voucher specimen of each sample was deposited in the Herbarium of the Laboratory of Natural Products (Faculty of Medicine and Pharmacy of Rabat). Each plant to be used for essential oil extraction was separately air-dried and ground. A sample of 200 g of powder from each plant was subjected to steam distillation for 2 h using a Clevenger-type apparatus recommended by the French Pharmacopoeia (Anonymous 1983). The yields were determined as grams over the 200 g of powder analysed in percentage, and are shown in Table 1. The oil was analysed in a Hewlett-Packard 5972 MS, fitted with a HP 5890 Series II GC and controlled by a G1034C Chemstation (Washington, USA). A sample of 1 μl was injected under the following conditions: column, DB 1 fused silica capillary column (20 m × 0.20 mm; film thickness 0.2 μm); carrier gas, helium (0.6 ml $min^{-1}$); injector temperature, 250°C; column temperature, from 50 to 250°C at 3°C $min^{-1}$; MS electronic impact 70 eV. Identification of the compounds was achieved by comparing retention times and mass spectra with those of the standards in the library (Stenhagen *et al*., 1974; Adams, 1995).

### Antifungal assay

Potato dextrose agar (PDA, Merck, Darmstadt, Germany) was used for growing *P. digitatum*, *G. citri-aurantii* and *B. cinerea;* V8 juice (Camden, NJ, USA) (a mixture of eight vegetable juices) for *P. citrophthora*. The media were autoclaved and cooled in a water bath to 40°C, each oil was added to sterilised water at a concentration of 1000 ppm and dissolved using an ultrasound homogeniser in an ice bath. Oil dispersion was facilitated by adding 0.2% Tween 80. The mixture was mixed with sterile molten PDA or V8 to obtain final concentrations of 0, 50, 150 and 250 ppm. The mixtures were then poured into Petri dishes (c.a. 20 ml per plate), which were seeded with a mycelial plug (5 mm diameter) from the edge of 7-day-old colonies of the test fungi. Three replicates for each treatment were kept at 24°C in the dark for 7 days, at which time the colonies on the control media had reached the edge of the dish. Growth inhibition was calculated as the percentage of radial growth inhibition compared with the control. The effect of the essential oils was compared with that of the synthetic fungicides: procymidone, propamocarbe HCl, guazatine and thiabendazole (TBZ). These fungicides were applied at a range of concentrations from 0.001 to 10 ppm. Two types of control dishes were used: one with medium only, the other with medium plus 0.2% Tween 80.

### *In vivo* trials

Essential oil from *C. viscidehirtum,* the most effective in the *in vitro* tests, was tested on clementine fruits (*Citrus reticulata* Blanco cv. Nules) collected directly from an unsprayed orchard. Fruits of uniform size, free from physical damage and disease symptoms, were selected. The fruits were disinfected in sodium hypochlorite (10%) for 3 min, washed three times with water and then inoculated by spraying with *P. citrophthora, P. digitatum* and *G. citri-aurantii* ($10^5$ conidia $ml^{-1}$ in 2% glucose containing a drop of Tween 80). The conidia concentration was determined using a Thoma slide. Inoculated fruits were randomly divided into groups of 6 fruits in 1.5 l plastic containers (10 replicates per treatment), contact time 3 h. *C. viscidehirtum* essential oil was dispersed in water without Tween 80 at 250, 1000 and 2000 ppm levels and stored in glass vials. After 3 h the mixtures were sprayed inside the containers. Control fruits were sprayed with distilled water. The boxes were sealed to saturate the air inside with volatiles. Clementine fruits were observed daily for symptoms, and the percentage of decayed fruits was determined at 3, 7 and 10-day intervals.

### Statistical analysis

Statystical analysis was performed applying the Newman-Keuls's test following the STATITCF statistical program.

## Results

The yield of the essential oils varied among species (Table 1). GC and GC-mass spectrum analyses of the oils permitted identification of the main components, which are listed in Table 1. *C. visci-*

Table 1. Chemical composition of essential oils from 25 Moroccan medicinal plants.

| Family | Plant species | Yield (%)[a] | Major constituents (%)[b] |
|---|---|---|---|
| Asteraceae | *Anthemis nobilis* L. | 1 | Camphor (12.9), chamazulene (12.6), β-caryophyllene (7.2), prochamazulene (7.1), α-terpineol (6), germacrene D (5.5) |
| | *Artemisia absinthum* L. | 2.5 | Isothyone (5.4), ar-curcumene (5.2), γ-elemene (2), linalool (4.6) |
| | *Artemisia herba alba* (Asso) | 0.3 | Camphor (46), α-thuyone (33.2), β-thuyone (9), camphene (8.5), 1,8-cineole (6.4) |
| | *Chrysanthemum viscidehirtum* (Schott) Thell | 0.2 | β-farnesene (25), limonene (21.8), sabinene (3.9), β-elemene (2.4), geraniol (3.1), caryophyllene oxide (2.4) |
| | *Chamomilla recutita* (L.) Ranschest | 1 | Chamazulene (12.6), camphor (12.9), β-farnesene (3.7) |
| Cupressaceae | *Cupressus sempervirens* L. | 0.2 | Sabenene (24.4), terpinene-4-ol (21.1), α-pinene (16.3), γ-terpinene (8), myrcene (5.1) |
| Geraniaceae | *Pelargonium* sp. Willd. | 0.2 | Citronellol (50), esters (25), geraniol (7), isomenthone (7) |
| Lamiaceae | *Calamintha officinalis* Moench | 1.5 | 1,8-cineole (36.6), pulegone (17.9), limonene (9.2), menthone (6.5) |
| | *Lavandula dentata* L. | 0.7 | Linalyl acetate (23.5), linalol (18.9), p-cymene (9.6) |
| | *Mentha piperita* L. | 2.5 | Menthol (50), menthone (30), methyl acetate (10) |
| | *Mentha pulegium* L. | 2.5 | Pulegone (85.4) |
| | *Ocimum basilicum* L. | 0.8 | 8-methyl-chavicol (85), linalol (3.4), 1,8-cineole (2.2) |
| | *Salvia aegyptica* L. Bria | 1.4 | Linalyl acetate (51.4), linalool (23), α-pinene (5.1) |
| Lauraceae | *Cinnamomum zeylanicum* Blume | 2 | Cinnamaldehyde E (61.2), α-terpineol (15), linalol (3.4) |
| Myrtaceae | *Eucalyptus globulus* Labill | 0.6 | 1,8-cineole (70.6), α-pinene (12.9) |
| | *Melaleuca viridiflora* Gaertin | - | 1,8-cineole (40.8), α-terpineol (32.1), limonene (5), β-pinene (3.1) |
| | *Myrtus communis* L. | 0.3 | α-pinene (37.6), 1,8-cineole (20), limonene (12), myrtenyl acetate (5) |
| Pinaceae | *Cedrus atlantica* Endl. | 0.3 | α-pinene (34.1), b-pinene (31.7), myrcene (17.2), limonene (5.1) |
| | *Pinus pinea* L. | 0.2 | α-pinene (37), β-pinene (3) |
| Ranunculaceae | *Nigella sativa* L. | - | p-cymene (50.2), thymoquinone (8.9), β-longipenene (4.4), E-anethole (3.8) |
| Rutaceae | *Citrus bergamia* Risso & Poiteau | 2 | Limonene (50), linalyl acetate (40), linalool (25) |
| | *Citrus lemon* Burm. | 0.5 | Limonene (66), geraniol (30.4) |
| | *Citrus sinensis* (L.) Osbeck. | 0.6 | Limonene (92) |
| | *Ruta chalepensis* L. | 1.1 | p-cymene (15.1), linalyl acetate |
| Verbenaceae | *Lippia citriodora* (Lam.) H.B.K. | 1.6 | Geraniol (15.4), spathulenol (13.1), nerol (11.9), limonene (10.1), 1,8-cineole (6.1), α-curcumene (5.9), caryophyllene oxide (5.6) |

[a] Grams of extracted oil as percentage over the 200 g of powder analysed from each plant.
[b] Grams over total extracted oil in percentage.

*dehirtum* essential oil mainly contained ß-farnesene, limonene and low percentages of sabinene, ß-elemene, geraniol and caryophyllene oxide. *Lippia citriodora* essence was mainly composed of geraniol, spathulenol and nerol. Essence of *C. viscidehirtum* showed the greatest inhibition of mycelial growth of all four fungi at the lowest concentration tested, with complete inhibition at 150 ppm after 7 days at 24±1°C (Table 2). *L. citriodora* and *Mentha pulegium* showed moderate antifungal

Table 2. Percent inhibition of radial growth of *Botrytis cinerea, Penicillium digitatum* and *Geotrichum citri-aurantii* on PDA, and of *Phytophthora citrophthora* on V8 medium when essential oils are added at different concentrations.

| Plant species | Essential oil concentration (ppm) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *B. cinerea* | | | *P. citrophthora* | | | *P. digitatum* | | | *G. citri-aurantii* | | |
| | 50 | 150 | 250 | 50 | 150 | 250 | 50 | 150 | 250 | 50 | 150 | 250 |
| *Artemisia herba-alba* | 0 e | 0 d | 0 e | 0 e | 0 f | 25.2 ef | 0 e | 0 f | 0 g | 0 d | 0 f | 0 g |
| *Chamomilla recutita* | 0 e | 0 d | 0 e | 0 e | 0 f | 1.9 g | - | - | - | - | - | - |
| *Anthemis nobilis* | 0 e | 0 d | 0 e | 0 e | 25.2 d | 41.6 d | - | - | - | - | - | - |
| *Chrysanthemum viscidehirtum* | 80 a | 100 a | 100 a | 76.6 b | 100 a | 100 a | 83.8 b | 100 a | 100 a | 88.5 b | 100 a | 100 a |
| *Cupressus sempervirens* | 0 e | 0 d | 0 e | 0 e | 0 f | 36.3 e | 0 e | 0 f | 0 g | 0 d | 0 f | 0 g |
| *Pelargonium* sp. | 6.3 de | 15.6 c | 18.9 d | 0 e | 23.8 d | 49.5 d | 0 e | 0.7 f | 10 f | 0 d | 15.2 d | 31.5 c |
| *Cinnamomum zeylanicum* | 0 e | 21.9 b | 24.8 d | 6.5 d | 24.3 d | 37.9 e | 0 e | 10.7 e | 31.9 d | 0 d | 13.7 de | 29.6 d |
| *Pinus pinea* | 0 e | 0 d | 7.4 de | 0 e | 31.9 c | 40.4 d | 0 e | 14 d | 21.5 e | 0 d | 17.1 d | 27.8 d |
| *Cedrus atlantica* | 11.9 d | 28.2 b | 44.4 bc | 0 e | 38 c | 45.4 d | 0 e | 13.7 d | 42.9 c | 0 d | 27.8 c | 43 b |
| *Eucalyptus globulus* | 0 e | 0 d | 2.2 e | 5.9 d | 29.6 d | 38.2 e | 0 e | 0 f | 1.9 g | 0 d | 0 f | 0 g |
| *Nigella sativa* | 0 e | 0 d | 0 e | 0 e | 0 f | 0 g | 0 e | 0 f | 0 g | 0 d | 0 f | 0 g |
| *Citrus bergamia* | 1.9 de | 22.2 b | 27 d | 0 e | 4.4 e | 20.4 ef | 0 e | 0 f | 0.7 g | 0 d | 0 f | 10 e |
| *Citrus lemon* | 0 e | 0 d | 4.1 de | 0 e | 0 f | 20.4 ef | 0 e | 0 f | 0 g | 0 d | 0 f | 0 g |
| *Citrus sinensis* | 0 e | 0 d | 3.7 e | 0 e | 0 f | 13 f | 0 e | 1.8 f | 31.9 d | 0 d | 6.3 e | 7 f |
| *Ruta chalepensis* | 19 c | 14.9 c | 34.1 c | 0.3 d | 27.6 d | 35.5 e | 6.3 de | 15.6 d | 18.9 f | 4.1 cd | 25.9 c | 28.2 d |
| *Lippia citriodora* | 3.7 de | 21.5 b | 69.3 b | 0 e | 34.1 c | 68.2 b | 11.9 d | 44.8 b | 44.4 c | 5.9 c | 31.1 b | 40.4 b |
| *Artemisia absinthum* | 0 e | 0 d | 0 e | 0 e | 0 f | 0 g | 0 e | 0 f | 0 g | 0 d | 0 f | 0 g |
| *Myrtus communis* | 0 e | 0 d | 0 e | 0 e | 0 f | 0 g | 0 e | 0 f | 0 g | 0 d | 0 f | 0 g |
| *Melaleuca viridiflora* | 0 e | 0 d | 0 e | 0 e | 0 f | 17.8 f | 0 e | 0 f | 0 g | 0 d | 0 f | 0 g |
| *Calamintha officinalis* | 0 e | 0.7 d | 16.3 | 0 e | 0 f | 44.4 d | 0 e | 0 f | 0 g | 0 d | 0 f | 0 g |
| *Lavandula dentata* | 0 e | 0 d | 1.9 e | 0 e | 0 f | 1.9 g | 0 e | 0 f | 0 g | 0 d | 0 f | 0 g |
| *Mentha piperita* | 0 e | 0 d | 0 e | 0 e | 0 f | 14 f | 0 e | 0 f | 0 g | 0 d | 0 f | 0 g |
| *Mentha pulegium* | 4.1 de | 30 b | 58.5 b | 0 e | 26 d | 33.3 e | 4.4 de | 31.5 bc | 51.9 b | 2.9 cd | 33.3 b | 40.7 b |
| *Ocimum basilicum* | 0 e | 0 d | 0 e | 0 e | 0 f | 12.6 f | 0 e | 0 f | 0 g | 0 d | 0 f | 0 g |
| *Salvia aegyptica* | 0 e | 0 d | 3.7 e | 0 e | 0 f | 3.7 g | 0 e | 0 f | 0 g | 0 d | 0 f | 0 g |
| Procymidone | 100 a | 100 a | 100 a | - | - | - | - | - | - | - | - | - |
| Propamocarbe HCl | - | - | - | 100 a | 100 a | 100 a | - | - | - | - | - | - |
| TBZ | - | - | - | - | - | - | 100 a | 100 a | 100 a | - | - | - |
| Guazatine | - | - | - | - | - | - | - | - | - | 100 a | 100 a | 100 a |
| Control | 0 e | 0 d | 0 e | 0 e | 0 f | 0 g | 0 e | 0 f | 0 g | 0 d | 0 f | 0 g |
| Control (Tween in distilled water) | 0 e | 0 d | 0 e | 0 e | 0 f | 0 g | 0 e | 0 f | 0 g | 0 d | 0 f | 0 g |

Means followed by the same letter in the same column are not significantly different according to the Newman-Keuls test (a=0.05).
-, not tested.

activity. *Cinnamomum zeylanicum, Cedrus atlantica, Citrus bergamia* and *Ruta chalepensis* essential oils at 250 ppm in that order reduced growth of the fungi from 44 to 25% (Table 2). Essential oils from *Nigella sativa*, *Artemisia absinthum, Lavandula dentata, Mentha piperita, Ocimum basilicum, Citrus lemon, Citrus sinensis* and *Myrtus communis* were not effective at doses lower than 250 ppm.

The antifungal effect *in vivo* of essence of *C. viscidehirtum* on clementine fruits inoculated artificially with *G. citri-aurantii, P. citrophthora* and *P. digitatum* was evaluated (Table 3). Symptoms appeared on treated fruits 7 days after treatment, while on the control fruits they appeared after only 3 days of storage. Exposure to *C. viscidehirtum* oil volatiles at 2000 ppm reduced brown rot, green mould and sour rot incidence by more than 77% after 10 days of storage (Table 3). No visible symptoms of phytotoxicity due to the oils was detected on the fruits.

Table 3. Percentage of rotted fruits (*Citrus reticulata* Blanco cv. Nules) after treatment with essential oil of *Chrysanthemum viscidehirtum* after 3, 7 and 10 storage days..

| Treatment | Concentration (ppm) | *P. citrophthora* | | | *P. digitatum* | | | *G. citri-aurantii* | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | 3 | 7 | 10 | 3 | 7 | 10 | 3 | 7 | 10 |
| *C. viscidehirtum* | 250 | 100 a | 100 a | 100 a | 100 a | 100 a | 100 a | 100 a | 100 a | 100 a |
| | 1000 | 100 a | 100 a | 100 a | 80.5 b | 100 a | 100 a | 90.3 b | 100 a | 100 a |
| | 2000 | 0 b | 12.5 b | 15.5 b | 0 c | 12.5 b | 22.2 b | 0 c | 7.9 b | 18.7 b |
| Propamocarbe HCl | 1000 | 0 b | 0 c | 0 c | - | - | - | - | - | - |
| TBZ | 1000 | - | - | - | 0 c | 0 c | 0 c | - | - | - |
| Guazatine | 1000 | - | - | - | - | - | - | 0 c | 0 c | 0 c |
| Control | | 100 a | 100 a | 100 a | 100 a | 100 a | 100 a | 100 a | 100 a | 100 a |

Means followed by the same letter in the same column are not significantly different according to the Newman-Keuls test (a=0.05). -, not tested.

## Discussion

*C. viscidehirtum* essential oil was found to contain ß-farnesene, limonene, geraniol and caryophyllene oxide. This oil provided very effective control of all fungi tested. Its effectiveness was due either to its high levels of ß-farnesene, limonene and sabinene, or to the fact that these compounds acted synergistically, as was suggested by Vampa *et al*. (1988). *C. viscidehirtum* also contained many oxygenated sesquiterpenes. Sesquiterpenes isolated from members of the Asteraceae possess a wide spectrum of biological activity (Marles *et al*., 1995) and appear to play a role in plant defence mechanisms. Wilson *et al*. (1987) found that a number of fruit volatiles (benzaldehyde) that emanate from peaches during ripening are fungicidal. Wilson *et al*. (1997) showed that fungitoxic compounds from plant essential oils can be used to control postharvest disease in fruits and vegetables. They also suggested that the natural plants could be used instead of methyl bromide as fumigants. It has been reported that most antifungal volatiles and vapors also reduce conidial germination, killing the fungi (Sholberg and Guance, 1995). Of the fungi, *P. citrophthora* was the most susceptible to the volatile compounds of all the essential oils tested. Müller-Riebau *et al*. (1997) reported that under greenhouse and plastic tunnel conditions 10 ml of essential oils from *Thymbra spicata* or *Saturja thymbra* in 50 g of perlite $m^{-2}$ of soil were effective against *Phytophthora capsici* in pepper. These findings were consistent with Bhaskara *et al*. (1998) who treated strawberry fruits with essence of *Thymus vulgaris*. It is concluded that essence of *C. viscidehirtum* is an antifungal agent that may have a role in the preservation of clementine fruits, which are very sensitive to infection from *B. cinerea*, *P. citrophthora, P. digitatum* and *G. citri-aurantii*.

## Acknowledgements

The authors wish to thank Professor A.M. Ben Tattou for identification of plant material.

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*Accepted for publication: October 10, 2003*