# Antifungal activities of extracts from selected Lebanese wild plants against plant pathogenic fungi

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**Summary.** Extracts of nine plant species growing wild in Lebanon were tested for their efficacy against seven plant pathogenic fungi: *Botrytis cinerea*, *Alternaria solani*, *Penicillium* sp., *Cladosporium* sp., *Fusarium oxysporum* f. sp. *melonis*, *Rhizoctonia solani* and *Sphaerotheca cucurbitae*. Extracts of three of the plants, *Origanum syriacum*, *Micromeria nervosa* and *Plumbago maritima*, showed the highest levels of *in vitro* activity against spore germination and mycelial growth of the fungi tested. *Inula viscosa* showed high activity against spore germination but only moderate activity against mycelial growth. The other five plant species tested *Calamintha origanifolia*, *Micromeria juliana*, *Ruta* sp., *Sideritis pullulans* and *Urginea maritima* showed only moderate to low activity against these fungi. Preventive sprays with extracts of *O. syriacum*, *M. nervosa, P. maritima* and *I. viscosa,* applied at concentrations ranging between 4 and 8% to squash and cucumber seedlings, gave efficient protection against gray mold caused by *B. cinerea* and powdery mildew caused by *S. cucurbitae*. However, these extracts did not control green mold of citrus fruits caused by *Penicillium* sp. Thin layer chromatography revealed three inhibitory bands in extracts of *O. syriacum*, two in *I. viscosa* and only one in each of the other plants tested: *M. nervosa*, *P. maritima*, *C. origanifolia* and *Ruta* sp.

**Key words:** antimycotic activity, *Inula, Micromeria, Origanum*, *Plumbago*.

## Introduction

With increasing awareness of environmental issues, the trend nowadays is towards developing sustainable agricultural ecosystems. Some key components of sustainability include integrated crop management (ICM) and organic agriculture. ICM and to a larger extent organic agriculture call for alternative approaches to pest management that reduce or eliminate the use of toxic pesticides.

Cultural, biological and physical control measures are integrated and pesticides can still be used but only when strictly necessary, and only when they respect the new natural approach. The major characteristics of such biorational pesticides are that they should have minimal toxic effects (acute or chronic) to humans and other non-target organisms, rapid degradation and often a narrow spectrum of activity. The indiscriminate use of pesticides, in addition to polluting the environment, has increased the problem of pest resistance to pesticides, which has become prominent since 1960 for arthropods and since the 1970s for plant pathogens (Loper *et al.*, 1991; Davis, 1994; Service, 1995). The trend towards environmentally friendly pes-

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ticides coupled with alarming levels of pest resistance to commonly used pesticides has lead to the search for new antimicrobial substances from various sources like medicinal plants (Clark, 1996; Cordell, 2000). In this context, various aromatic plants have been tested *in vitro* for their antibacterial and antifungal properties (Jeyarajan *et al.*, 1987; Deans and Svoboda, 1990; Zambonelli *et al.*, 1996; Falerio *et al.*, 1999; Abou-Jawdah *et al.*, 2002). The main objective of the present study was to search for Lebanese wild plants with strong *in vitro* and *in vivo* antifungal activity that may be used for the management of plant diseases caused by fungi.

In a previous study (Abou-Jawdah *et al.*, 2002), nine plant species were evaluated for their effectiveness against a range of serious plant diseases. This study investigated seven more wild plants for the same purpose, in addition to wild marjoram and inula, already evaluated in the earlier study. The antifungal properties of *Micromeria nervosa* and *Plumbago maritima* are reported for the first time in this study.

## Materials and methods

### Plant material and extraction

Samples were collected from wild plants (Table 1) mostly at the flowering stage. Fifty grams of air-dried leaves from each plant species except *Plumbago maritima,* for which bulbs were used, were cut into small pieces, macerated with 250 ml of methanol, shaken for 30 min and incubated overnight at 4°C. The extracts were then filtered through a sintered glass funnel and the residue was extracted twice in the same manner. Methanol extracts were pooled and roto-evaporated at 40°C to reduce the volume to 100 ml. An equal volume of methanol was added and the mixture stored overnight at -20°C to separate waxy materials. The mixture was filtered, then roto-evaporated as above, the oil fractions were collected with ethanol and the final volume was brought to 5 ml and the material stored at -20°C until use. All solvents used were of analytical grade.

### Micro-organisms

All the fungi were isolated at the American University of Beirut from infected plant organs. *Botrytis cinerea*, *Alternaria solani*, *Penicillium* sp., and *Cladosporium* sp., cause flower, fruit or seed rots; *Fusarium oxysporum* f. sp. *melonis* vascular wilts and *Rhizoctonia solani* mainly root rots. *Sphaerotheca cucurbitae,* which causes powdery mildew on cucumber, is an obligate parasite and was excluded from the *in vitro* tests.

### Antimycotic tests

The toxicity of plant extracts against six of the fungi was tested using a poisoned dish technique. Plant extracts diluted in ethanol were added to the autoclaved culture medium (Difco [Kansas, MS, USA] potato dextrose agar (PDA), 15 ml/plate) when the temperature of the medium reached about 50°C. Extract and PDA were mixed thoroughly. The final concentration was adjusted to 2 ml of extract in 98 ml of PDA (2%), the equivalent to 20 g of air dried material 100 ml$^{-1}$. Unamended PDA dishes and dishes amended with ethanol at concentrations equal to those used with the extracts served as controls. For the calculation of the minimum inhibitory concentration (MIC) and the $ED_{50}$ the following plant extract concentrations were used: 0.015, 0.03, 0.06, 0.125, 0.250, 0.5, 1.0 and 2.0%, and the $ED_{50}$ values were calculated from log dosage-response curves.

Table 1. Scientific name, common name and family of plant species used in this study.

| Scientific name | Family | Common name |
| --- | --- | --- |
| *Calamintha origanifolia* (Labill.) Boiss. | Labiatae | Calamint |
| *Inula viscosa* (L.) Ait. | Compositae | Inula |
| *Micromeria juliana* (L.) Benth. | Labiatae | Savory |
| *M. nervosa* (Desf.) Benth. | Labiatae | Emperor's mint |
| *Origanum syriacum* Sieb. exs. et L. | Labiatae | Wild marjoram |
| *Plumbago maritima* (L.) | Plumbaginaceae | Leadwort |
| *Ruta* sp. | Rubiaceae | Rue |
| *Sideritis pullulans* Vent. | Lamiaceae | Ironwort |
| *Urginea maritima* (L.) Bak. | Asphodelaceae | Sea onion |

*Mycelial inhibition tests*

Mycelial growth inhibition tests were performed by placing 5 mm mycelial agar disks, cut from the margin of expanding fungal colonies, in the centre of PDA dishes amended with plant extract or unamended. The colony diameter was measured after incubating for 7 days in the dark at 22ºC. All treatments were replicated three times and repeated at least once. Percent inhibition was calculated after subtraction of the diameter of the initial inoculum disk.

*Spore germination tests*

Spore suspensions ($10^6$ ml$^{-1}$) of four fungi (Table 2) were prepared from actively growing colonies (7 to 11 days old). Ten $\mu$l drops of spore suspensions were added to PDA dishes amended with a plant extract or with the ethanol control. Dishes were incubated at 22ºC until spore germination in the control reached >70% (6–15 h according to the rate of germination of each fungus), and then a drop of 37% formaldehyde was added to the medium to inhibit further germination and development of the germ tubes. A spore was considered germinated when the length of the germ tube was more than twice the length of the spore. Three replicates were conducted for each treatment, and a minimum of 200 spores were counted in each replicate. Percent inhibition was calculated according to Abbott's formula: (% germinated in control - % germinated in treatment / % germinated in control $\times$ 100).

**Chromatography**

Thin layer chromatography (TLC) using silica gel plates (Fluka # 99570, 0.25 mm, Steinheim, Switzerland) was performed by spotting 5–35 $\mu$l of concentrated plant extracts on the silica plates. The following solvent systems were tried: chloroform:methanol (9:1, v:v), n-propanol:ethyl acetate (7:3, v:v), and three mixtures of ethyl acetate:petroleum ether (7:3, 5:1, 1:9, v:v). For sequential (bidirectional) chromatography, chloroform:methanol was followed by n-propanol:ethylacetate. After the development of the chromatograms, the silica plates were dried in a forced-air oven at 37ºC for 1 h. Each silica plate was cut in half. One half of the plate was used for the bioassay by spraying the developed TLC plate with 2 ml of potato dextrose broth containing a spore suspension ($10^6$ ml$^{-1}$) of *Cladosporium* sp. used as test organism. Zones of inhibition were detected visually after incubation at 100% relative humidity at room temperature for 3–5 days. Three replicates were conducted. From the other half of the TLC plate, zones corresponding to each inhibition zone were scraped off, eluted with absolute ethanol, collected into an Eppendorf tube and centrifuged at 1000 $g$ for 15 min. The supernatant was used for another TLC separation (sequential) using a different solvent system. The bioassay test was repeated and zones corresponding to the inhibition bands were eluted and used for GC-MS analysis after evaporation of the solvent and dilution with ethyl acetate or hexane.

Table 2. Antifungal activity of plant extracts as determined by mycelial growth inhibition tests.

| Plant extract | Inhibition of mycelial growth[a, b] (%) | | | | |
|---|---|---|---|---|---|
| | *Botrytis cinerea* | *Alternaria solani* | *Cladosporium* sp. | *Fusarium oxysporum* f. sp. *melonis* | *Rhizoctonia solani* |
| *Calamintha origanifolia* | 66 | 14 | 54 | 48 | 21 |
| *Inula viscosa* | 70 | 63 | 67 | 65 | 65 |
| *Micromeria juliana* | 22 | 12.5 | 30 | 22 | 18 |
| *M. nervosa* | 100 | 100 | 100 | 100 | 100 |
| *Origanum syriacum* | 100 | 100 | 100 | 100 | 100 |
| *Plumbago maritima* | 100 | 100 | 100 | 100 | 100 |
| *Ruta* sp. | 33 | 42 | 70 | 66 | 65 |
| *Sideritis pullulans* | 77 | 45 | 65 | 62 | 74 |
| *Urginea maritima* | 3 | 15 | 19 | 8 | 0 |

[a] Data collected after 7 days of incubation.

[b] Average of three replicates, referring to extraction done with methanol and diluted with pure ethanol.

Eluted fractions from the inhibition zones of the TLC plates were chromatographed and analyzed using a GS-MS gas chromatograph as reported previously (Abou-Jawdah *et al.*, 2002).

### *In vivo* tests

*In vivo* tests were performed on squash and cucumber seedlings and on freshly harvested lemon fruits.

#### *Preparation of inoculum*

Spore suspensions of *B. cinerea* or *Penicillium* sp. were harvested from cultures grown on PDA by adding 10 ml of sterilized water containing 0.1 M glucose and 0.07 M $KH_2PO_4$. The suspensions were filtered through several layers of sterile muslin cloth and the spore concentration was adjusted to the desired level.

#### *Penicillium green mold.*

Lemons were washed with chlorox (10%) and pricked with a needle in three places to a depth of 3 mm. The needle pricks were treated by applying 50 $\mu$l of plant extract (concentrations 4 to 8%) or fungicide at the recommended rate and were left to dry at room temperature for 2 h. Three inoculum concentrations of *P. digitatum* were tested ($10^5$, $0.5 \times 10^6$ and $10^6$ $ml^{-1}$). Inoculated fruits were incubated at 10 or 20°C. Data on the percentage of puncture-wounds infected and on the diameter of rot lesions were recorded after 3–7 days (Smilanick *et al.*, 1999; Karabulut *et al.*, 2002).

#### *Powdery mildew*

Cucumber seedlings were sprayed until run off with plant extracts, fungicides, or a solution of wetting agent as control (Table 7). Freshly collected cucumber leaves infected with *S. cucurbitae* were tapped at the top of a 1 m high cone covering the cucumber seedlings at the 2-leaf stage. Each treatment was replicated six times. Inoculated seedlings were kept in a glasshouse at ambient conditions. The percentage of leaf area covered with fungal growth was estimated one week after inoculation.

#### *Gray mold*

Squash seedlings were sprayed as mentioned above (Table 8). After drying for 2 h at room temperature, each cotyledon was inoculated with 10 $\mu$l of a spore suspension of *Botrytis cinerea* ($10^6$ $ml^{-1}$).

Each treatment was replicated 6 times using one seedling per replicate and the experiment was repeated twice. Inoculated seedlings were kept in a growth chamber (Microclima Snijders Scientific Model 1750, Tilburg, Netherlands) where the relative humidity was maintained at 100% for 48 h, then lowered to 95%. The night/day temperature was regulated at 18–22ºC. Disease incidence and lesion diameters were recorded five days after inoculation (Abou-Jawdah and Itany, 1995).

### Statistical analyses

Statistical analyses were conducted using MSTAT–C/ANNOVA 1 for all parameters in the tests listed above. Orthogonal contrast was accomplished and all tests of significance were conducted at $P \leq 0.05$.

## Results

### Antimycotic tests

Preliminary screening of plant extracts at a concentration of 2% showed that four of the plant species tested: *P. maritima*, *M. nervosa*, *I. viscosa* and *O. syriacum,* had a high level of efficacy against all the pathogenic fungi tested (Tables 2 and 3) with about 100% control of spore germination of *A. solani*, *B. cinerea*, *Cladosporium* sp. and *F. oxysporum* f. sp. *melonis*. Similar results were achieved with mycelial inhibition, except for *I. viscosa,* which was less effective as a mycelial inhibitor. These four plant species were selected for further evaluation. Among the other plants tested *Ruta* sp. and *S. pullulans* showed moderate activity, with over 60% inhibition of at least three of the fungi tested; while the antifungal activity of *M. juliana*, *C. origanifolia* and *U. maritima* was low. In general, inhibition of spore germination was higher than inhibition of mycelial growth (Tables 2 and 3).

*Botrytis cinerea* and *Cladosporium* sp. were selected to determine the MIC and the $ED_{50}$ of the plant extracts (Table 4). Extracts of *O. syriacum* showed the highest efficacy, with a MIC of 0.03% to 0.06% on spore germination and mycelial growth of both fungi. *M. nervosa* had slightly lower activity, with a MIC of 0.125% on spore germination and 0.5% on mycelial growth of both fungi. *P. maritima* was more effective against spore germination of *Cladosporium* sp. (MIC 0.06%) than against that of *B. cinerea* (MIC 0.5%), while the MIC on myceli-

Table 3. Antifungal  activity of plant extracts as determined by spore germination inhibition tests.

| Plant extract | Inhibition of spore germination[a, b] (%) | | | |
| --- | --- | --- | --- | --- |
| | *Botrytis cinerea* | *Alternaria solani* | *Cladosporium* sp. | *Fusarium oxysporum* f. sp. *melonis* |
| *Calamintha origanifolia* | 41 | 50 | 58 | 51 |
| *Inula  viscosa* | 100 | 89 | 100 | 97 |
| *Micromeria  juliana* | 35 | 22 | 40 | 36 |
| *M. nervosa* | 100 | 98 | 100 | 100 |
| *Origanum syriacum* | 100 | 99 | 100 | 99 |
| *Plumbago  maritima* | 100 | 100 | 100 | 100 |
| *Ruta* sp. | 65 | 49 | 80 | 73 |
| *Sideritis  pullulans* | 80 | 65 | 72 | 66 |
| *Urginea  maritima* | 15 | 27 | 34 | 26 |

[a] Calculated according to Abbot's formula.
[b] Average of three replicates referring to extraction done with methanol and diluted with pure ethanol.

Table 4. Antifungal activity of plant extracts: determination of minimum inhibitory concentration and $ED_{50}$. Values expressed as percentage dilution of plant extracts.

| Plant extract | *Cladosporium* sp. | | | | *Botrytis cinerea* | | | |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| | Spore germination | | Mycelium growth | | Spore germination | | Mycelium growth | |
| | MIC[a] | $ED_{50}$[b] | MIC | $ED_{50}$ | MIC | $ED_{50}$ | MIC | $ED_{50}$ |
| *Micromeria nervosa* | 0.125 | 0.015 | 0.500 | 0.060 | 0.125 | 0.086 | 0.500 | 0.350 |
| *Origanum syriacum* | 0.060 | 0.021 | 0.060 | 0.042 | 0.030 | 0.021 | 0.060 | 0.042 |
| *Inula viscosa* | 1.017 | 0.180 | >2.000 | 0.250 | 2.000 | 0.250 | >2.000 | 1.001 |
| *Plumbago maritima* | 0.060 | 0.021 | 2.000 | 0.701 | 0.500 | 0.250 | 1.000 | 0.500 |

[a] Minimum inhibitory concentration leading to 100% inhibition of spore germination and mycelium growth.
[b] Plant extract effective  concentration leading to  50% inhibition.

al growth ranged between 1–2% for both fungi. *Inula* was the least effective with a MIC of 1–2% on spore germination, and over 2% on mycelial growth.

**Chromatography**

The number of inhibition zones, their $R_F$ values and the size of the inhibition area varied among the plant extracts. Among all solvents used, chloroform:methanol (9:1, v:v) and ethyl acetate:petroleum ether (1:9, v:v) showed improved separation with sharper fungal inhibition zones after inoculation and incubation of the TLC plates at 37°C for 3–4 days in humidity chambers. Between 5 and 10 $\mu$l of each of the plant extracts tested was sufficient to produce inhibitory zones in both these solvent systems. With the ethyl acetate:petroleum ether solvent system, *O. syriacum* and *I. viscosa* each produced two inhibition zones, while *M. nervosa, P. maritima*, *S. pullulans*, *C. origanifolia* and *Ruta* sp. had one inhibitory zone each (Tables 5 and 6). Similar results were obtained with chloroform:methanol except that *O. syriacum* showed three inhibitory zones (Tables 5 and 6). In this last system the bands were too close and had a higher $R_F$ value.

The natural products present in the inhibition zones on the TLC plates were eluted and analyzed by GC-MS. Preliminary analysis showed that thymol, carvacrol, naphthalene and phenanthrene may have represented some of the major active compounds in *O. syriacum*. In *Inula* the major active compounds were not identified; however, phenathrene and an azulene derivative were detected

Table 5. Antifungal activity of plant extracts: $R_F$ values of fungal inhibition zones obtained following chromatography on TLC plates[a] using two solvent systems and inoculation of TLC plates with *Cladosporium* sp.

| Plant extract | $R_F$ of inhibition zones[b] | |
| --- | --- | --- |
| | ethyl acetate:petroleum ether (1:9, v/v) | chloroform:methanol (9:1, v/v) |
| *Calamintha origanifolia* (35 µl) | 0.34–0.45 | 0.61–0.67 |
| *Inula viscosa* (35 µl) | 0.32–0.51 | 0.55–0.66 |
| | 0.55–0.72 | 0.67–0.81 |
| *Micromeria nervosa* (25 µl) | 0.65–0.83 | 0.70–0.89 |
| *Origanum syriacum* (35 µl) | 0.64–0.79 | 0.55–0.65 |
| | 0.85–0.99 | 0.70–0.80 |
| | | 0.85–0.90 |
| *Plumbago maritima* (35 µl) | 0.67–0.96 | 0.70–0.89 |
| *Ruta* sp. (35 µl) | 0.17–0.35 | 0.25–0.50 |

[a] The volume of plant extract used on the TLC plates is indicated in parentheses.
[b] $R_F$ values are shown based on the lower and upper border of the inhibition zone; this was done because some of the inhibition zones were quite large.

Table 6. Antifungal activity of plant extracts: $R_F$ values of fungal inhibition zones obtained following sequential (bidirectional) chromatography on TLC plates[a]. $R_F$ values in n-propanol:ethyl acetate represent the first separation while those in chloroform:methanol represent the second separation of the corresponding inhibition zones.

| Plant extract | $R_F$ of inhibition zone [b] | | | | | |
| --- | --- | --- | --- | --- | --- | --- |
| | n-propanol:ethyl acetate (70:30, v:v) | | | chloroform:methanol (9:1, v:v) | | |
| | 5 µl | 10 µl | 20 µl | 5 µl | 10 µl | 20 µl |
| *Inula viscosa* | 0.11-0.13 | 0.14-0.16 | 0.16-0.20 | 0.81-092 | 0.83-0.93 | 0.85-0.94 |
| | 0.26-0.48 | 0.26-0.50 | 0.25-0.52 | 0.94-0.98 | 0.91-0.98 | 0.94-0.99 |
| *Micromeria nervosa* | 0.48-0.72 | 0.48-0.76 | 0.52-0.80 | 0.73-0.95 | 0.73-0.97 | 0.74-0.98 |
| *Origanum syriacum* | 0.16-0.28 | 0.14-0.29 | 0.16-0.36 | 0.54-0.63 | 0.53-0.68 | 0.55-0.70 |
| | 0.35-0.75 | 0.32-0.84 | 0.38-0.80 | 0.73-0.75 | 0.70-0.77 | 0.71-0.78 |
| | | | | 0.81-0.90 | 0.81-0.99 | 0.80-0.98 |
| *Plumbago maritima* | 0.65-0.88 | 0.71-0.98 | 0.76-0.95 | 0.73-0.95 | 0.75-0.94 | 0.80-0.91 |

[a] Fluka, silica plates #99570, 0.25 mm.
[b] $R_F$ values are shown based on the lower and upper border of the inhibition zone; this was done because the inhibition zones were quite large.

at low concentrations. In *Micromeria*, a phthalate and a quinazoline compound were identified in addition to low concentrations of menthone and diphenyl sulfone.

### *In vivo* tests
### Green mold

All plant extracts even at the maximum concentrations used (8%) failed to prevent infection of lemon fruits by *Penicillium* sp., which reached 100% in all extract treatments, even at the lowest spore concentration ($10^5$ spores ml$^{-1}$) and at both incubation temperatures (10°C or room temperature). Fruit rot developed on inoculated fruits within 3–6 days of inoculation. The fungicides used in the same experimental protocol gave 100% control of infections. Phytotoxic symptoms on lemon fruits were observed with *Plumbago* extract at 8%.

Table 7. Efficacy of plant extracts to prevent powdery mildew on cucumber.

| Plant extract | Average proportion of leaf area covered with powdery mildew[a,c,d] |
|---|---|
| Control | 0.83 a |
| Control +wetting agent[b] | 0.50 bc |
| Carbendazim (0.06 g 100 ml^{-1}) | 0.32 d |
| Chlorothalonil (0.25 g 100 ml^{-1}) | 0.53 b |
| Iprodione (0.3 g 100 ml^{-1}) | 0.03 e |
| *Origanum syriacum* 4% | 0.35 d |
| *O. syriacum* 8% | 0.14 e |
| *Inula viscosa* 4% | 0.30 d |
| *I. viscosa* 8% | 0.15 e |
| *Plumbago maritima* 4% | 0.37 cd |
| *P. maritima* 8% | 0.35 d |
| *Micromeria nervosa* 4% | 0.63 b |
| *M. nervosa* 8% | 0.00 e |

[a] Average of six replicates.
[b] Wetting agent $F_4/F_6$, 75 $\mu$l/75 $\mu$l .
[c] Average amount of leaf area infected.
[d] Means in the same column followed by the same letter do not differ significantly ($P < 0.05$) according to DMRT.

Table 8. Efficacy of plant extracts to prevent gray mold on squash.

| Plant extract | Number of infected cotyledons[a]/out of 12 inoculated |
|---|---|
| Control | 9 a [b] |
| Control + wetting agent[c] | 8 a |
| Carbendazim (0.06 g 100 ml^{-1}) | 0 b |
| Chlorothalonil (0.25 g 100 ml^{-1}) | 0 b |
| Iprodione (0.3 g 100 ml^{-1}) | 0 b |
| *Inula viscosa* 4%[d] | 8 a |
| *I. viscosa* 8% | 0 b |
| *Micromeria nervosa* 4% | 1 b |
| *M. nervosa* 8% | 1 b |
| *Origanum syriacum* 4% | 2 b |
| *O. syriacum* 8% | 0 b |
| *Plumbago maritima* 4% | 0 b |
| *P. maritima* 8% | 0 b |

[a] Number of infected cotyledons over total number of inoculated cotyledons.
[b] Means in the same column followed by the same letter do not differ significantly ($P < 0.05$) according to DMRT, following arcsin transformation considering every 4 cotyledons as a replicate.
[c] Wetting agent $F_4/F_6$ 75 $\mu$l/75 $\mu$l.
[d] Plant extract diluted with control solution + wetting agent.

*Powdery mildew*

This assay showed some variability among the same replicates inoculated with *S. cucurbitae* and was therefore repeated four times. The general trend showed that the plant extracts had moderate to high efficacy in controlling powdery mildew. Results of a representative trial are shown in Table 7. *M. nervosa*, *O. syriacum* and *I. viscosa* at the highest concentration used (8%) gave high levels of control, as did iprodione, and were more effective than carbendazim or chlorothalonil.

*Gray mold*

Three to 4 days after inoculation with *B. cine-*

*rea*, cotyledons that had been sprayed with the wetting agent or with water showed similar levels of gray mold infection. Extracts of *P. maritima, O. syriacum* and *M. nervosa* at concentrations of 4% or more showed good efficacy in preventing gray mold, similar to that of the fungicides iprodione, chlorothalonil and carbendazim at the recommended concentrations. With *I. viscosa,* on the other hand, a concentration of 8% was required to prevent infection.

## Discussion

Previous studies reported that *O. syriacum* and *I. viscosa* had antifungal activity (Mehta *et al.*, 1984; Deans and Svoboda, 1990; Bourrel *et al.*, 1993; Adam *et al.*, 1998; Abou-Jawdah *et al.*, 2002). In this study the antifungal effectiveness of *O. syriacum* and *I. viscosa* was compared with that of extracts from seven other wild plant species, for whose antifungal activity no or only scant information was available.

Among the seven other plant species tested for use in the management of plant pathogenic fungi, *P. maritima* and *M. nervosa* showed high efficacy, approaching that of *O. syriacum,* and better than that of *I. viscosa*. In the mycelial inhibition tests, extracts of *O. syriacum, M. nervosa* and *P. maritima* showed high efficacy, with 100% inhibition of the fungi tested. The MICs of *P. maritima, M. nervosa* and *I. viscosa* extracts controlling both *Botrytis cinerea* and *Cladosporium* sp. were higher than that of *O. syriacum* (from 0.5 to 2% respectively, compared with 0.06% for *O. syriacum*). These three plant species had a similar activity against spore germination of *Cladosporium,* with an $ED_{50}$ of about 0.015–0.02%, but they varied in their efficacy against *B. cinerea*, with *I. viscosa* being less effective than *M. nervosa* and *O. syriacum* ($ED_{50}$=0.25% for *I. viscosa,* compared with 0.09 and 0.21%, for *M. nervosa* and *O. syriacum* respectively).

Little is known about the antifungal activity of the other plants tested: *S. pullulans, Ruta* sp., *M. juliana* and *C. origanifolia*, which in this study had moderate to low activity against the five phytopathogenic fungi. The essential oils of these species were studied previously (Ojala *et al.*, 2000; Hernandez-Perez and Rabanal, 2002). In this study *S. pullulans* extracts showed moderate activity against four of the five fungi studied and therefore may have a

use in combination with other molecules to strengthen their activity. Different species of *Sideritis* (Lamiaceae), widespread in the Canary Islands, are employed in folk medicine as cytostatic, antimicrobial, vulnerary, astringent agents, as a flu vaccine and as a circulatory stimulant (Darias *et al.*, 1990).

Plants that exhibited the highest *in vitro* activity were tested *in vivo* and found to prevent infection with the gray mold fungus *B. cinerea*, and with the powdery mildew fungus *S. cucurbitae* under simulated field conditions. However, the need for high concentrations of these extracts (4–8%), may prevent their practical use in the field. The high concentrations required may be due to the rapid inactivation, volatilization and/or degradation of the active principles in these extracts upon exposure to environmental conditions. The identification of the major active components may be a step towards deriving more active or more stable products from these plants

The major essential oils previously reported in the four Labiatae species tested in this study were thymol and/or carvacrol in *O. syriacum* (Falerio *et al.*, 1999; Abou-Jawdah *et al.*, 2002), menthone, and pulegone in *Calamintha* sp. (Couladis and Tzakou, 2001; Kitic *et al.*, 2002a, b; Hidalgo *et al.*, 2002) In *Micromeria* sp. the major essential oil was borneol, piperitenone oxide or pulegone depending on the species (Stojanovic *et al.*,1999; Tabanca *et al.*, 2001). In the Lamiaceae the only genus studied was *Sideritis* and the major compound previously reported in almost all species was α-pinene (Baser *et al.*,1997; Kirimer *et al.*, 2001; Ozkan *et al.*, 2001; Tzakou, 2002). In the Plumbaginaceae, all studies focus on plumbagin and its effect on carcinoma cells in animals, but there is no information about the composition of the essential oils (Ali-Shtayeh *et al.*, 1998).

In many of the cases studied, antifungal activity was associated with a compound that occurred in low quantity but possessed high biological activity; the occurrence of such compounds may be masked by a high concentration of other compounds. This study used a bioassay with sequential (bidirectional) chromatography (n-propanol:ethyl acetate followed by chloroform:methanol) in order to achieve a better partial purification of the active compounds. *O. syriacum* showed three inhibition bands in the

chloroform:methanol elution, and two in ethyl acetate:petroleum ether. *Inula* showed two inhibition zones, and the three other plants tested showed only one inhibitory band each. Comparison of the $R_F$s of the inhibitory zones in both solvent systems (Table 6) showed clearly that in the six plants tested there were at least 9 compounds or families of compounds with antifungal activity. Identification of these compounds would be very desirable since some may belong to a new chemical group or may exhibit a new mode of action, thus representing new antifungal compounds that can be used either alone or in mixtures. Some of these potential active compounds, especially those derived from *Origanum* and *Micromeria,* may be used in organic agriculture or in ICM very easily since these two plants have been used for human consumption or as medicinal plants in Lebanon for centuries. Our preliminary GC-MS analysis of *O. syriacum* extracts is in general agreement with previous reports stating that thymol and carvacrol are major antimicrobial compounds, but the bioassay proved that they were only a small part of the major active compounds in *O. syriacum*. For the other plants studied, even though some compounds were identified at low concentrations, further analysis would be highly desirable, using different techniques including HPLC-MS and NMR, to identify the active principles. Proper identification of these principles may lead to the synthesis of new, more stable analogues with improved efficacy, as was the case with the relatively new class of fungicides, the strobilurins, which were derived from fungi.

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## Literature cited

Abou-Jawdah Y. and H. Itany, 1995. Sensitivity of *Botrytis cinerea* isolates to fungicides used in Lebanon. *Phytopathologia Mediterranea* 34, 100–108.

Abou-Jawdah Y., H. Sobh and A. Salameh, 2002. Antimycotic activities of selected plant flora, growing wild in Lebanon, against phytopathogenic fungi. *Journal of Agricultural and Food Chemistry* 50, 3208–3213.

Adam K., A. Sivropoulou, T. Kokkini Lanaras and M. Arsenakis, 1998. Antifungal activities of *Origanum vulgare* subsp. *hirtum*, *Mentha spicata*, *Lavandula angustifolia*, and *Salvia fructicosa* essential oils against human pathogenic fungi. *Journal of Agricultural and Food Chemistry* 46, 1739–1745.

Ali-Shtayeh M.S., R.M. Yaghmour, Y.R. Faidi, K. Salem and M.A. Al-Nuri, 1998. Antimicrobial activity of 20 plants used in folkloric medicine in the Palestinian area. *Journal of Ethnopharmacology* 60, 265–271.

Baser K.H.C., N. Kirimer and G. Tumen, 1997. Essential oil of *Sideritis scardica* Griseb. subsp. *scardica*. *Journal of Essential Oil Research* 9, 205–207.

Bourrel C., G. Vilarem and F. Perineau, 1993. Chemical analysis, bacteriostatic and fungistatic properties of the essential oil of elecampane (*Inula helenium* L.) *Journal of Essential Oil Research* 5, 411–417.

Clark A.M., 1996. Natural products as resource for new drugs. *Pharmaceutical Research* 13, 1133–1141.

Cordell G.A., 2000. Biodiversity and drug discovery - A symbiotic relationship. *Phytochemistry* 55, 463–480.

Couladis M. and O. Tzakou, 2001. Essential oil of *Calamintha nepeta* subsp. *glandulosa* from Greece. *Journal of Essential Oil Research* 13, 11–12.

Darias V., L. Bravo, R.M. Rabanal, C.C. Sánchez-Mateo and D.A. Martín-Herrera, 1990. Cytostatic and antibacterial activity of some compounds isolated from several *Lamiaceae* species from the Canary Islands. *Planta Medica* 56, 70–72.

Davis J., 1994. Inactivation of antibiotics and the dissemination of resistance genes. *Science* 264, 375–382.

Deans S.G. and K.P. Svoboda, 1990. The antimicrobial properties of marjoram (*Origanum origanum* L.) volatile oil. *Flavour and Fragrance Journal* 5, 187–190.

Falerio L., G.M. Miguel, C.A.C. Guerrero and J.M.C. Brito, 1999. Antimicrobial activity of essential oils of *Rosmarinus officinalis* L., *Thymus mastichina* (L.) spp. *mastichina* and *Thymus albicans* Hofmanns and Link. *Acta Horticulturae* 501, 45–48.

Hernandez-Perez M. and R. Rabanal, 2002. Evaluation of the anti-inflammatory and analgesic activity of *Sideritis canariensis* var. *pannosa* in mice. *Journal of Ethnopharmacology* 81, 43–47.

Hidalgo P.J., J.L. Ubera, J.A. Santos, F. Lafont, C. Castellanos, A. Palomino and M. Roman, 2002. Essential oils in *Calamintha sylvatica* Bromf. ssp. *ascendens* (Jordan) P.W. Ball: wild and cultivated productions and antifungal activity. *Journal of Essential Oil Research* 14, 68–71.

Jeyarajan R., S. Doraiswamy, R. Bhaskaran and S. Jayaraj, 1987. Effect of neem and other plant products in the management of plant diseases in India. In: *Natural Pesticides from the Neem Tree (*Azadirachta indica *A. Juss) and other Tropical Plants*. (H. Schmutterer, K.R.S. Ascher, ed.), *Proceedings of the Third International Neem Conference,* Nairobi, Kenya, 1986, Deutsche Gesellschaft für Technische Zusammenarbeit (GTZ), Eschborn, Germany, 703 pp.

Karabulut O., L. Cohen, B. Wiess, A. Daus, S. Lurie and S. Droby, 2002. Control of brown rot and blue mold of peach and nectarine by short hot water brushing and yeast antagonists, *Postharvest Biology and Technology* 24, 103–111.

Kirimer N., N. Tabanca, K.H.C. Baser and G. Tumen, 2001. Composition of the essential oil of *Sideritis congesta* P.H. Davis et Hub-Mor. *Journal of Essential Oil Research* 13, 132–133.

Kitic D., T. Jovanovic, M. Ristic, R. Palic and G. Stojanovic, 2002. Chemical composition and antimicrobial activity of the essential oil of *Calamintha nepeta* (L.) Savi ssp. *glandulosa* (Req.) P.W. Ball from Montenegro. *Journal of Essential Oil Research* 14, 150–152.

Kitic D., R. Palic, G. Stojanovic and M. Ristic, 2002. Composition of the essential oil of *Calamintha vardarensis* (Greuter et Burdet) Silic. *Journal of Essential Oil Research*, 14, 58–59.

Loper J.E., M.D. Henkels, R.G. Roberts, G.G., Grove, M.J. Willett and T.J. Smith, 1991. Evaluation of streptomycin, oxytetracycline, and copper resistance of *Erwinia amylovora* isolated from pear orchards in Washington State. *Plant Disease* 75, 287–290.

Mehta B.K., A. Batra and S.C. Mehta, 1984. *In vitro* antimicrobial efficiency of *Inula* oil against gram-positive, gram-negative human pathogens and phytopathogenic fungi. *Indian Perfumer* 28, 123–125.

Ojala T., S. Remes, P. Haansuu, H. Vuorela, R. Hiltunen, K. Haahtela and P. Vuorela, 2000. Antimicrobial activity of some coumarin containing herbal plants growing in Finland. *Journal of Ethnopharmacology* 73, 299–305.

Ozkan M., J.C. Chalchat and A. Akgul, 2001. Essential oil composition of Turkish mountain tea (*Sideritis* spp.). *Food Chemistry* 75, 459–463.

Service R.F., 1995. Antibiotics that resist resistance. *Science* 270, 724–727.

Smilanick J.L., D.A. Margosan, F. Mlikota, J. Usall and F. Michael, 1999. Control of citrus green mold by carbonate and bicarbonate salts and the influence of commercial postharvest practices on their efficacy. *Plant Disease* 83, 139–145.

Stojanovic G., I. Palic, J. Ursic-Jancovic, V. Vajs and D. Dokovic, 1999. Chemical composition of the essential oil of *Micromeria albanica* (Griseb. ex K. Maly) Silic. *Journal of Essential Oil Research* 11, 785–787.

Tabanca N., N. Kirimer, B. Demirci, F. Demirci and K.H.C. Baser, 2001. Composition and antimicrobial activity of the essential oils of *Micromeria cristata* subsp. *phrygia* and the enantiomeric distribution of borneol. *Journal of Agricultural and Food Chemistry* 49, 4300–4303.

Tzakou O., 2002. The essential oil of *Sideritis raeseri* Boiss. et Heldr. ssp. *attica* (Heldr.) Pap. et Kok. *Journal of Essential Oil Research* 14, 376–377.

Zambonelli A., A. Zechini D'Aulerio, A. Bianchi and A. Albasini, 1996. Effects of essential oils on phytopathogenic fungi *in vitro*. *Journal of Phytopathology* 144, 491–494.