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Phytochemical effects of phytoplasma infections on essential oil of *Monarda fistulosa* L.

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Abstract

Two essential oils hydrodistilled from the aerial parts of phytoplasma-infected plants of *Monarda fistulosa* L. (wild bergamot) growing at the Herb Garden of Casola Valsenio (Emilia Romagna Region; Italy), have been analyzed and compared by GC and GC/MS. The first oil was extracted from wild bergamot plants showing phytoplasma symptoms (virescence, yellows and stunting) and infected by aster yellows and stolbur phytoplasmas; the second oil was distilled from asymptomatic plants in which stolbur phytoplasmas were identified. These two oils were similar in composition to a third oil, of Italian origin, from *M. fistulosa* cultivated in Trentino Region. The oil from plants infected with aster yellows plus stolbur infected plants showed an increase in the quantity of some monoterpenes, as well as in the content of α-caryophyllene and a marked decrease in the content of thymol; the oil from symptomless material (in which stolbur phytoplasmas were detected) yielded a significant increase in thymol concentration and a marked decrease in monoterpene compounds.

Key words: wild bergamot, PCR, RFLP, phytoplasmas, essential oils, GC/MS.

Introduction

Monarda fistulosa L. ("wild bergamot") is an annual or perennial medicinal plant known for its strong therapeutic effects: its essential oil is characterized by high antibacterial, antimycotic, and anti-inflammatory activities and, for this reason, has been recently proposed for the treatment of seborrhoea (Zhilyakova *et al*., 2009).

Figure 1. Flowers of *Monarda fistulosa*: symptomless (a) and symptomatic (b).

During a survey carried out in 2009 in Italy, wild bergamot showing yellows, stunting, virescence and flower bud proliferation, was found for the first time, to be infected by a phytoplasma belonging to 16Sr subgroup 16SrXII-A "stolbur" (Bellardi *et al*., 2011). In 2010, a study to verify the correlations among the presence of phytoplasma, symptom expression, and the effects of these prokaryotes on essential oil composition was carried out.

Materials and methods

During Summer 2010, molecular tests to confirm the presence of phytoplasma were carried out in *M. fistulosa* plants belonging to the same crop surveyed in 2009, (Herb Garden, Casola Valsenio, Italy). Starting in July, increasing percentages of plants showing phytoplasma symptoms (more than 80%) were observed. Symptomless (=SLP) and symptomatic plants (=SP) (figure 1) were labelled by visual inspection of their aerial parts and tested at blooming stage to verify phytoplasma presence and to determine their identity. After a chloroform/phenol extraction (Prince *et al*., 1993), plants were tested by direct PCR with primers P1/P7 followed by nested PCR with primers R16F2/R2 (Lee *et al*., 1995). RFLP analyses were performed with *Tru*I and *Tsp509*I for 16 hours at 65°C and with *Hha*I for 16 hours at 37°C and analysed on 5% polyacrilamide gels after ethidium bromide staining.

About 900 gr fresh aerial part material of SP and about 120 gr of SLP were collected in August at the end of flowering, and hydrodistilled; the two oils were separated from water and kept in tightly closed amber vials before analyses. Identification of the compounds was made by combined gas chromatography mass spectrometry (GC/MS) and by comparison of retention times of *M. fistulosa* components with those of a control oil from plants grown in Trentino region (Northern Italy) (table 1).

Table 1. Composition of *M. fistulosa* oils.

Compound	% Content Symptomless (SLP)	Symptomatic (SP)	Control	R_i
α-Thujene	0.40 ± 0.03	4.05 ± 0.11	2.60 ± 0.08	923
α-Pinene	0.38 ± 0.03	3.04 ± 0.08	2.36 ± 0.07	942
β-Pinene	1.60 ± 0.04	10.92 ± 0.21	3.10 ± 0.10	978
Myrcene	4.91 ± 0.21	3.92 ± 0.11	8.05 ± 0.13	986
α-Phellandrene	2.78 ± 0.16	8.60 ± 0.31	13.70 ± 0.21	1003
β-Phellandrene	9.96 ± 0.33	14.42 ± 0.13	17.02 ± 0.27	1005
p-Cymene	12.38 ± 0.25	14.86 ± 0.24	13.49 ± 0.19	1024
Δ^3-Carene	2.92 ± 0.12	0.84 ± 0.03	3.95 ± 0.09	1027
β-Terpineol	0.89 ± 0.053	0.51 ± 0.03	0.44 ± 0.02	1137
α-Terpineol	2.00 ± 0.11	0.80 ± 0.04	0.61 ± 0.04	1178
Carvacrol methyl ether	9.83 ± 0.31	9.08 ± 0.22	3.51 ± 0.09	1205
Thymol	43.57 ± 0.55	20.79 ± 0.43	26.48 ± 0.39	1282
α- Caryophyllene	0.27 ± 0.01	2.65 ± 0.09	0.40 ± 0.02	1425
β-Caryophyllene	2.90 ± 0.09	1.07 ± 0.05	0.69 ± 0.03	1428
Epibicyclosesquiphellandrene	1.25 ± 0.09	0.49 ± 0.03	0.22 ± 0.01	1431
Germacrene D	3.16 ± 0.18	3.22 ± 0.10	2.44 ± 0.09	1486
γ-Cadinene	0.35 ± 0.04	0.47 ± 0.02	0.19 ± 0.01	1504
δ-Cadinene	0.45 ± 0.03	0.27 ± 0.02	0.75 ± 0.03	1508

Results and discussion

In 2009, only 50% of the plants showed phytoplasma symptoms and were found to be infected only by stolbur, while in 2010, phytoplasmas were detected from both symptomless and symptomatic plants (there were no phytoplasma-free plants). Both direct and nested PCR, as well as RFLP analyses on 16Sr DNA seqeunces confirmed that in all SPL wild bergamot plants were infected by stolbur phytoplasma and SP plants were infected by mixed phytoplasmas: stolbur and another phytoplasma belonging to ribosomal subgroup 16SrI-B (Aster yellows, '*Candidatus* Phytoplasma asteris': AY). Considering that every single plant, with or without symptoms, was phytoplasma-infected, no phytoplasma-free material has been collected to obtain control oil from healthy plants. The increasing of phytoplasma infection (100%) inside the Herb Garden occurred as a consequence of the presence of leafhoppers, weeds and other medicinal phytoplasma-infected plants, such as *Echinacea purpurea* Moench, *Digitalis lutea* L., *D. lanata* Ehrh., *Grindelia robusta* L., recently reported as "new natural host" of AY and/or stolbur phytoplasmas (Bellardi *et al*., 2007).

Differences in compositions between the oils from SP and SLP were observed. As shown in table 1, in which the percentage presence of 18 components is listed according to their elution order, the oil from SP showed an increase in the quantity of some monoterpenes like α-thujene, α- and β-pinene, and β-phellandrene as well as in the content of α-caryophyllene and a marked decrease in the content of thymol. On the other hand, oil from SLP yielded only a significant increase in thymol concentration as the only relevant modification and a marked decrease in monoterpene compounds.

Composition of the oils obtained by distillation varies significantly among different *M. fistulosa* hybrids (Mazza and Marshall, 1992); thymol accounted for c.a. 20% of the oil from SP, but over 40% of that from SLP. The thymol reduction in mixed phytoplasma infected plants has particular relevance since tymol is one of the main constituents of the plants having antibacterial and antimycotic activity. Further studies are in progress to compare therapeutic effects of the studied essential oils.

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