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Chemical response of parsley and mentha herbs to certain stress agents

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Abstract

In response to certain stress agents, infection with *Cercospora petroselini* and treatment of this infection with Cuprosan, changes in the quantity and quality of the volatile oils produced from *Mentha piperita* L. and *Petroselinum Crispum* Mill become apparent. The treated samples showed increase in the concentration of Cu ions in the leaves. Some components were produced under the effect of these stress agents (infection and treatment). Psoralen was isolated from samples treated with Cu salts. Corylidin, Angladin and Pereflorin B were isolated from Parsley herb under the influence of *Cercospora petroselini* infection. These compounds were examined for antimicrobial activity. Corylidin, Angladin and Psoralen, inhibited growth of *Pseudomonas putida* , *E. Coli* and *Rhizobium meloloti* (gm−ve bacteria) , while Pereflorin B inhibited *Streptococcus lactis* and *Bacillus subtilis* (gm+ve bacteria).

Keywords: *Mentha piperita* L.; *Petroselinum crispum* Mill; Stress with *Cercospora petroselini* and cuprosan

1. Introduction

Fungitoxic compounds are produced by many plants in response to inoculation with fungi or treatment with abiotic agents (Preston, 1975; Ingham & Harborne, 1976; Martin & Dewick, 1979) . The foliar treatment of the infected plant with Cu salts, causes increase in the volatile oil content during vegetative and floral stages of growth (El-Sherbeny & Abou-Zied, 1986). Some air pollutants such as sulphur pollution (SO_2) increased volatile oil content because they increase synthesis of some S-amino acids as methionine (Mahran, Saleh, Fathy, Motawe, & Hashem, 1995).

Heavy metals, such as Cu salts, are still being prescribed in traditional herbal medicines. In recent years intensive studies on the role and toxicity of trace heavy metals in humans have been carried out (Puves, 1985; Tamita, 1990). The increased awarness of the toxicity of these heavy metals, have resulted in a number of reports of heavy metal poisoning from ingestion of herbal medicines, so the limits of Cu must not exeed 25 ppm (Wong, Tan, & Wee, 1993).

2. Materials and methods

2.1. Plant material

Seeds of Parsley and cuttings of *Mentha piperita* L. produced from the Egyptian Ministry of Agriculture were sown in the greenhouse of the National Research Centre Farm on 10 March 1996. The young plants, after appearance of leaves, were subjected to infection on 24 May 1996 by spraying with *Cercospora petroselini* and then covered with plastic sheets for 3 days; infection was repeated again after 15 days.

Samples were taken for analysis before infection, and after 3, 7 and 10 days from the second inoculation. This infection was treated twice, 15 days apart by Cuprosan 311 SD (Rhône. Poulenc, Agrochimie) which contains 30% Cu metal. Samples were taken for analysis after 4 and 7 days from the first treatment and after one and two weeks from the second treatment. Samples taken at each time were analysed freshly for volatile oils (quantitative and qualitative). GC/MS analyses were carried out for volatiles using a gas chromatograph directly coupled to a mass spectrophotometer, Finnigan (USA), with flame ionization detector. Capillary column was DB-5 fused silica, 30 m length, 0.25 mm i.d. and 0.25 μm

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thickness; carrier: Helium at 30 ml min^{-1}; temp. prog.: 60–220°C at a rate of 3°C min^{-1}; chart speed: 0.5 cm min^{-1}; ion source temp.: 180°C ionization voltage: 70 eV.

The identification of the constituents was performed by comparison of their mass spectral fragmentation with that of the published data (Adams, 1989; Mass Spectrometry Data Centre, 1974), while quantitative determination was carried out based on peak area measurments. The amounts of Cu in the collected samples were determined according to Chapman and Pratt, (1961) and Cottenie, Verloo, Kiekens, Velghe and Camerlynch, (1982) by digesting the dry herb and measuring Cu concentration with an Atomic Absorption Spectrophotometer (Pye Unicam Sp 1900 UK).

Some of the fresh samples were macerated in 95% ethanol for phytochemical analysis. The ethanol extract was evaporated under reduced pressure at 40°C; the residue was successively extracted with pet. ether, ether, chloroform, ethyl acetate and then methanol.

Each extract was subjected to chromatographic analysis on silica gel plates using chloroform: methanol, 9:1 and benzene: acetone, 9:1 and compared with control samples.

Three components were isolated from the infected plants (compounds I, II and III) while one compound (IV) was isolated from plants treated with Cuprosan.

These compounds were examined by spectral analysis for identification and examined for antimicrobial activity by using pure strains of microbes which were kindly obtained from the Microbial Genetics Department, NRC, Egypt. (*Bacillus subtilis* CAIM1007, *Streptococcus lactis* DSM20250, *E.Coli* HB101, *Pseudomonas putida* ABM45 and *Rhizobium meloloti*). Tests were carried out using the diffusion assay method and nutrient agar as medium (Hammond & Lambert, 1978). The diameter of cups made was 0.8 cm on which to put a specified amount of the tested substances, and concentrations used were 0.5 g% ampicillin (Wyeth, England), and 10 mg ml^{-1} of each component, dissolved in 10% Tween 80 (which was used as a blank in each plate). The capacity of the cup was 0.02 ml. Zones of inhibition were determined in each case (see Table 5 below).

3. Results and discussion

From Table 1, it was found that the infected plants, Mentha and Parsley herbs, showed a marked decrease in their volatile oil contents. This was confirmed before by (Mahran, Saleh, Fathy, Motawe, & Hashem, 1995); when plants were exposed to certain damage, a decrease in volatile oil production was produced. But when these infected plants were treated by Cu salt once, they retained their health state and oil production increased. Applying Cu salt without previous infection to the plants, increased their volatile oil production; this is consistent with the observations of El-Sherbeny and Abou-Zied, (1986).

When Cu salt treatments were repeated 15 days apart, oil production decreased; therefore, when plants are infected, Cu salts must be used in moderate amounts or they may adversly affect the plants.

Tables 2 and 3 show the quality of volatile oils produced after the infection and treatment of these herbs. The value of Mentha oil is measured according to its contents of menthone and menthol; it is obvious from Table 2 that the infected plants showed a decrease in menthol percentage from 43.8 to 41.89%, while samples which were subjected to Cuprosan only showed a decrease in menthone from 27.35 to 24.92% and increase in menthol percentages from 43.8 to 46.58%. These effects were intensified in plants which were exposed to the two stress agents (infection and treatment); menthone decreased to 10% and menthol increased to 56.2%.

Parsley herb confirmed this behaviour of Mentha, the major components in it are myristicin and 1,3,8 para menthatriene, changed completely in case of the infected, infected and treated, and the treated plants from the control one.

Myristicin which is a phenol ether increased very much in infected plants (81.5%) and to some extent, in treated-infected (76.3%) and in treated non-infected plants (67.6%) while the control was 39.7%. 1,3,8 Para menthatriene, which is a cyclic terpene, showed a distinct decrease, especially in infected plants (4.05%), treated-infected decreased 2.13% and treated non-infected, 3.25%,while the control was 17.1%. Some other components decreased such as α-pinene, myrcene, globulol and dill apiole. This was confirmed earlier by Rizk, Hammoda, Lashin, Nofal, Azam, and Wood, (1983) and Rizk, Hammoda, Pohland, Wood, Abdallah, Saleh, Lashin, and Nofal, (1983) who showed that the phenolic compounds were produced due to fungal infection.

Cu increased very much in the leaves of mentha and parsley, (Table 4). So Cu showed its highest concentration in plants which were treated twice, 15 days apart, and its concentration decreased gradually with time in mentha (92, 75, 66 and 50 ppm, respectively, one week apart). In Parsley the values were 86, 73, 60 and 45 ppm respectively. It is clear that after one month from applying Cuprosan (twice) for treatment, concentration of Cu is more than the permitted levels in edible plants (25 ppm), while using it only once gave acceptable concentrations of Cu metal, in Mentha (27, 22, 18 and 15 ppm, respectively) and in Parsley (25, 20, 12 and 10 ppm, respectively) in relation to the control. Cu is a very important element for plants; it is taken up as Cu^{2+} and Cu^{-} chelates from the soil and incorporated

Table 1
Effect of Cuprosan on the percentage of volatile oil content (v/w) of fresh leaves of *Mentha piperita* L. and *Petroselinum crispum* Mill., infected and non-infected by *Cercospora pertroselini*

Time	Control		Plants treated with Cuprosan		Plants infected with *C. petroselini*					
					Non-treated		Plants treated with Cuprosan			
							One field application rate		Double field application rate	
	M	P	M	P	M	P	M	P	M	P
*Before infection	0.50	0.1			0.50	0.1				
*Apply infection:										
After 3 days					0.46	0.08				
After 7 days					0.46	0.06				
After 10 days					0.42	0.06				
*Apply first treatment:										
After 4 days			0.60	0.08	0.41	0.06	0.60	0.08		
After one week			0.60	0.12	0.40	0.05	0.48	0.1		
*Apply second treatment:										
After one week			0.60	0.09	0.39	0.06	0.50	0.09	0.48	0.08
After two weeks			0.50	0.12	0.40	0.06	0.49	0.11	0.40	0.12

M: means Mentha herb.
P: means Parsley herb.

Table 2
Effect of infection with *C. petrselini* and Cuprosan treatment on the percentage components of volatile oil of *Mentha piperita* L.

Component	Control	Plants treated with Cuprosan	Plants infected with *C. petroselini*	
			Non-treated	Treated with Cuprosan
α-Pinene	0.11	0.44	0.09	0.01
β-Pinene	0.20	0.05	0.14	0.01
Δ-3-Carene	1.27	0.89	0.90	0.34
Limonene	0.08	0.74	0.01	0.01
Cineole	3.41	3.99	3.86	1.76
Octanol	0.16	0.13	0.16	0.16
Menthone	27.34	24.9	27.1	10.0
Isomenthone	4.16	4.71	4.68	2.71
Linalool	0.56	0.47	0.65	1.47
Menthyl acetate	4.52	2.91	3.64	5.00
Neomenthol	4.27	3.47	4.28	4.48
β-Caryophyllene	0.62	0.86	0.44	0.32
Isopulegol	0.57	0.44	0.50	0.56
Menthol	43.8	46.60	41.9	56.2
Humulene	0.31	0.23	0.22	0.12
α-Terpeniol	0.34	0.39	0.30	0.40
Germacrene D	0.62	0.75	0.53	0.51
Piperitone	0.43	0.11	–	0.01

Table 3
Effect of infection with *C. petroselini* and Cuprosan treatment on the percentage components of volatile oil of *Petroselinum crispum* Mill

Component	Control	Plants treated with Cuprosan	Plants infected with *C. petroselini*	
			Non-treated	Treated with Cuprosan
α-Pinene	6.94	2.39	3.05	2.39
Camphene	0.11	–	0.02	–
β-Pinene	4.57	3.62	2.02	3.62
Myrcene	23.8	15.5	4.29	15.5
α-Phellanderene	1.26	1.86	0.37	1.86
Cymenen-para	0.86	1.60	1.64	1.60
Menthatriene 1,3,8-para	17.1	3.25	4.05	3.25
Cis-α-farnesine	1.94	1.64	0.59	1.64
Myristicin	39.7	67.6	81.5	67.6
Globulol	1.11	0.98	0.06	0.98
Dill apiole	4.03	1.10	0.70	1.10
Bisabolol (epi-alpha)	0.71	0.51	0.07	0.51

in plants as complexes and components of enzymes (plasto-eyanin and phenol oxidases). It has a function in metabolism (photosynthesis and oxidases) (Larcher, 1983), but must be controlled as a heavy metal, when eaten as food, causes renal failure, blood count deseases and liver problems.

Some coumarins have been isolated from plants under the influence of certain fungi or heavy metals (Saleh, El.Olemy, Hemaia, El-Shami, & Saleh, 1983), acting as fungitoxic compounds. From the chromatographic study of the successive extracts of infected and treated samples of Parsley herb, three compounds were isolated from infected samples and one compound was isolated from treated samples, which were not present in the control plant. Carylidin, a coumesterol was isolated from the pet. ether extract, Angladin, an ester of coumarin was present in pet. ether and chloroform extracts while Pereflorine B was isolated frorn chloroform extract of infected Parsley herb. Psoralen was isolated from a chloroform extract of treated Parsley herb. From the preparative chromatography on silica gel G6 using benzene: acetone (9:1) or benzene: ethyl acetate (9:1), these compounds were isolated and purified.

Table 4
Concentration of Cu metal (ppm) in mentha and parsley leaves infected with *C. petroselini* and treated with cuprosan

Time	Control		Plants treated with cuprosane					
			non-infected		Plants infected with *C. petroselini*			
					One feild application rate		Double feild application rate	
	M	P	M	P	M	P	M	P
After one week	9	7	27	30	27	28	92	85
After two weeks	9	7.7	25	21	22	20	75	73
After three weeks	8.5	7	19	10	18	12	66	60
After four weeks	9	7	12	7	15	10	50	45

M: means Mentha herb.
P: means Parsley herb.

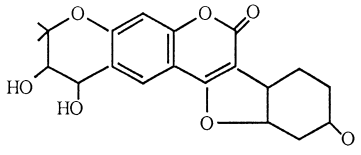
3.1. Compound I

This was isolated from the pet. ether extract of the infected sample at R_f0.25 with blue florescence under UV at 336 nm, as white crystalline needles with mp 300°C; it gives no ferric chloride reaction but yellow colour was obtained with aq. NaOH. λ max values in ethanol were 201, 232, 239, 275, 334 nm. In a KBr disk, IR gave peaks at 3360, 3210, 3100, 2980, 1701, 1635, 1610, 1575, 1495, 1450, 870 cm^{-1}.

Proton NMR at 200 MHz in DMS0 gave δ at 1.13(s), 1.17 (s), 4.27 (d.J = 4.5 Hz), 5.29 (br), 6.89 (d.d. J = 8.1, 2.1 Hz), 6.99 (s), 7.12 (d.J = 2.1 Hz), 7.65 (d.J = 8.1 Hz), 7.86 (s).

MS showed prominent peaks at m/e (rel. int.) 368 (M^+, 100%). 355 (96%), 308 (24%), 296(98%), 293 (98%), 292 (95%), 281 (12%), 69 (43%), 59 (95%), 43 (92%).

These data are the same as those of Carylidin with molecular formula $C_{20}H_{16}O_7$ and structural formula

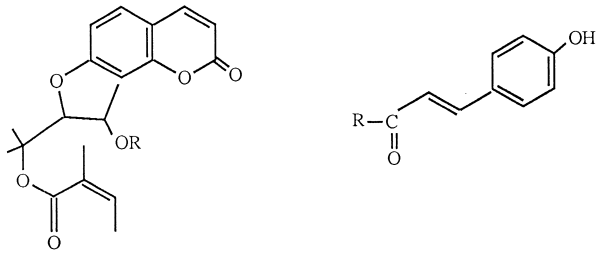


Compound I

3.2. Compound II

This was present in the pet. ether and chloroform extracts of infected samples, isolated at R_f0.4 as colourless needles, mp 156–158°C. λ max values in ethanol were 231, 240, 275 and 326 nm. IR gave peaks at 3450, 1700, 1720, 1600, 1580, 1510 cm

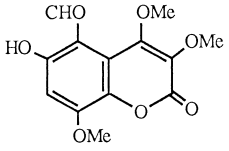
MS at *m/e* (rel. int.): 490 (M^+, 10%), 226 (100%), 198 (72%), 164 (28%), 147 (22%), 120 (18%), 100 (20%), 83(18%), 55 (20%). So Compound II had all the characteristics of Angladin with molecular formula $C_{28}H_{26}O_8$ and structural formula



Compound II

3.3. Compound III

This was isolated from the chloroform extract of infected samples with a yellow colour under UV and mp. 199–200°C, R_f 0.22. λ max values were 207, 216, 239, 394 nm. MS at 70 eV, *m/e* (rel. intensity), 280 (M^+, 5%), 256 (38%), 213 (7%), 185 (5%), 129 (6%), 67 (7%), 60 (28%), 55 (32%), 43 (66%), 41 (100%). Compound III indicates pereflorine B with molecular formula $C_{13}H_{12}O_7$ and structural formula



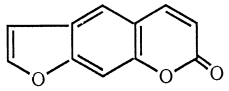
Compound III

3.4. Compound IV

This was isolated from the pet. ether and chloroform extracts of Parsley herb treated with Cu salts (Cuprosan) with a blue colour under UV at 336 nm, R_f 0.32, λ values in methanol were 232, 268, 415, 428 nm. MS, *m/e* (rel. int.) 186 (M^+, 100%). 158(80%), 149(13%), 130(21%), 102(35%), 76(18%).

^{1}HNMR at 200 MHz in deuterated methanol gave δ at 6.36 (d.J = 9.8 Hz), 8.08 (d.J = 9.8 Hz), 7.96(s), 7.54 (d.J = 1.0 Hz), 7.02 (d.d. J = 2.5 Hz, 1.0 Hz), 7.95 (d.J = 2.5 Hz).

Compound IV was found to have the molecular formula $C_{11}H_6O_3$ and the structural formula of psoralen



Compound IV

When these compounds were examined for antimicrobial activity (Table 5) it was found that compounds

Table 5
Effect of Components I, II, II and IV isolated from parsley herb, under the stress of infection and treatment, on some gm + ve and gm − ve bacteria

Organism	Zone of inhibition in cm				
	Compound I	Compound II	Compound III	Compound IV	Amp.
Gm − ve bacteria					
Streptococcus lactis	–	–	2.0	–	3.0
Bacillus subtilis	–	–	1.8	–	2.5
Gr − ve bacteria					
Pseudomonas patida	1.2	1.4	–	1.4	1.7
E.Coli	1.2	1.6	–	1.4	2.5
Rhizobium meloloti	1.5	1.6	–	2.0	2.2

I, II IV inhibit growth of *E. Coli*, *Pseudomonas putida* and *Rhizobium meloloti* while compound III inhibits *Streptococcus lactis* and *Bacillus subutilis*.

Mentha piperita L. has been previously studied under the influence of the heavy metal, lead, as an air pollutant and metahydroxy cinnamic acid was then produced (Mahran, Saleh, Fathy, Motawe, & Hashem, 1995).

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