Qualitative properties of *Mentha* × *piperita* (L.) after application of the fungicide Hattrick DP-50

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ABSTRACT

The aim of this work was to study and verify the effect of the Hattrick DP-50 fungicide on quality and content of essential oil in *Mentha* × *piperita* (L.) HUDS. Cv. Perpeta (peppermint) during different developmental phases. The fungicide was applied approximately one month before the first collection. The results have shown that the phase of full bloom may be regarded as a period with the highest content of essential oil in herb and leaves of peppermint; its greatest amount was found in the youngest leaves. The content of menthol gradually increased to its maximum value in the full bloom phase, while that of menthone was decreasing. The treatment with Hattrick DP-50 did not influence the essential oil content and its changes during vegetation when compared to untreated plants. Similarly, the application of fungicide did not cause detectable changes in relative representation of main and secondary components of the essential oil.

Keywords: 1,8-cineol; limonene; composition of terpenes; tebuconazole and tolylfluanid; plant signal pathway

The most important commercial essential oil-producing species is peppermint (*Mentha* × *piperita*), a perennial herb of the family Lamiaceae. *Mentha* × *piperita* is indigenous in Europe and has widespread in cultivation throughout all regions of the world. The essential oil is obtained from the fresh leaves of peppermint by steam distillation and it offers a promising potential for future applications within the fields of agriculture, medicine, pharmaceutical industry and biotechnology. Fairly high consumption of the herb and leaves of peppermint as a drug along with increasing demands of the pharmaceutical industry laid on the amount and quality of the essential oil require ensuring continuous production of this plant. The economic variability of the peppermint oil industry depends upon maintaining high average yields of top quality oil. The rust presents a serious problem in the cultivation of commercial peppermint crops. The rust diseases are the most devastating diseases effecting aromatic and medicinal plants. They cause leaves to wither and fall, and also reduce the yield affecting thus the quality of essential oils and biologically active substances (Parbery 1996). Rust diseases affected over 50% of the leaves; however, a prolonged infection can almost completely defoliate the plant (Margina and Zheljazkov 1995). Mint rust is caused by the *Puccinia menthae* Pers. fungus. It attacks many members of the herb family Lamiaceae, including peppermint. If uncontrolled, mint rust reduces oil yield by 50% or more and also reduces the quality and quantity of oil produced by commercially grown peppermint (Edwards et al. 1999). The control of mint rust is thus very important. Edwards et al. (1999) showed that failure to use any treatment to control rust resulted in death of mint plants within four years. Nonchemical means of control, such as cultivation or the use of flame, were used, but generally the use of fungicides is reasonably effective.

The research we have conducted is aimed at systematical studies of the composition of essential oils in various medicinal plants (Farkas et al. 2003, Mistrikova and Vaverkova 2009) with a special focus on the influence of pesticides on the composition of these essential oils (Vaverkova et al. 1997). Essential oils from *Mentha* × *piperita* exhibit a high level of antifungal activity and they are comparable to synthetic fungicides (Farkas et al. 2003, Hadian et al. 2008). Still, adapted or resistant pathogenic species are capable to strongly influence the yield values; an optimal fungicide (Hattrick DP-50) must be thus sought in order to protect the cultivated vegetation.

Our work is directed towards the evaluation of the effect of Hattrick DP-50 on the quality and amount of the essential oil from peppermint in its main development phases, which are interesting for pharmaceutical industry. Used Hattrick DP-50 has two active ingredients tolylfluanid and tebuconazole (40:10). Tolylfluanid (classes: N-trihalomethylthio; chemical name: 1,1-dicloro-N-[(dimethylamino)sulfonyl]-1-fluoro-N-(4-methylphenyl)methanesulfenamide) and tebuconazole (classes: azole and/or triazole; chemical name: (RS)-1-(4-chlorophenyl)-4,4-dimethyl-3-(1*H*-1,2,4-triazol-1-ylmethyl)pentan-3-ol. Tolylfluanid is non-phytotoxic when used as direct and in plants it is rapidly hydrolysed to DMST which is further hydrolysed and conjugated. Tebuconazole inhibits ergostanol biosynthesis.

MATERIAL AND METHODS

The experimental material was *Mentha* × *piperita* (L.) var. Perpeta grown at the standard agrochemical conditions in the Nitra locality. The plant material was collected in the main developmental phases: (a) bifurcation, (b) bud set, (c) beginning of bloom, (d) full bloom and (e) fade of blooming. The plants were collected from small stands of control plants and stands treated with Hattrick DP-50 (tebuconazole/tolylfluanid – 10/40). Postmergent application of the fungicide was done in the phase of development of the first four leaves; it was approximately one week before the first collection. The above ground part of plants, i.e. herb and leaves were dried at 32–34°C. The results of analysis were recalculated to the dry matter. The essential oil from samples was distilled off by the method according to the Slovak pharmaceutical codex (1997). Then it was analysed by gas chromatography.

Analysis of the oil samples was done using a Hewlett Packard HP 5971A mass selective detector directly coupled to an HP 5890 Series II FID gas chromatograph. A fused silica capillary column Carbovax 20M (30 m × 0.32 mm I.D., df 0.2 μm); Qaudrex Corp. New Haven, Connecticut, U.S.A., was used. The temperature programme was as follows: temperature of column was held at 60°C for 2 min, then 4°C/min to 220°C for 15 min; temperature of injector 210°C, temperature of detector 250°C; carrier gas N 1 ml/min, injected sample 0.2 μl (microsyringe Hamilton, U.S.A.). Mass spectra were recorded at ionisation energy (EI) of 70 eV.

Oil components were identified by comparison of their mass spectra with those from databases NBS 75K, INRA MASS (LRSA Dijon France), Wiley 138, and NIST.

RESULTS AND DISCUSSION

The properties of essential oil from peppermint, one of the most important spice plants, were studied and the essential oil components were determined using gas chromatography. In order to find out the effect of Hattrick DP-50 on some qualitative properties of peppermint we investigated the content of essential oil and its composition during vegetation period in the herb and leaves of treated and untreated (control) peppermint plants. These parameters were evaluated in relation to the main developmental phases and growth of the plants.

Peppermint produces high levels of p-menthane monoterpenes including menthone and menthol (Lawrence 1981). During leaf development, the total content of monoterpenes undergoes significant change (Burbott and Loomis 1969, Croteau and Martinkus 1979, Maffei et al. 1986, Brunn

Table 1. Percentage of the main and some secondary components of the essential oil from the above-ground parts of untreated peppermint plants in individual developmental phases

	(–)-menthol		(–)-menthone		(–)-menthyl-acetate		mentho-furane		1,8-cineole		(–)-limonene	
Onthogenesis phase	A	B	A	B	A	B	A	B	A	B	A	B
Bifurcation	29.0	32.1	37.9	38.0	2.0	3.3	0	0	1.5	2.0	4.7	3.9
Bud set	46.2	45.3	29.1	28.9	4.4	5.6	1.4	0	2.9	2.1	4.3	4.0
Beginning of bloom	55.4	54.8	22.1	21.6	6.6	6.3	4.4	1.4	5.7	4.6	3.4	3.4
Full in bloom	58.4	57.6	16.5	12.0	7.8	6.4	5.7	3.4	7.9	6.4	1.8	1.8
Fade of bloom	58.0	56.1	12.1	11.1	8.2	7.3	7.1	5.1	9.0	6.6	0.4	1.4

et al. 1991). The results summarised in Tables 1 and 2 indicate that the menthol content increased at given conditions during vegetation period in both untreated and treated plants, and reached its maximum in the full bloom phase. Simultaneously with the increase of the menthol content a decrease of the menthone content occurred. From the moment of bud set or beginning of bloom phase, the occurrence of menthofurane was recorded and its content gradually increased. Content of menthyl acetate and 1,8-cineole increased during vegetation and their maximum was observed at the fade of blooming, while an opposite tendency was observed for the limonene content, which decreased at the end of blooming. Thus, from the moment of creation of the reproductive organs a decrease of menthone content was observed accompanied by a strong increase of the menthol content. Treatment with Hattrick DP-50 did not cause any significant changes in the quality of essential oil. The differences found in the values of its main components are negligible.

Based on the obtained results, it may be stated that peppermint contains the greatest amount of the essential oil in the full in bloom phase. This maximum was observed for both chemically treated and control plants. In the fade of bloom phase the plant stops its growth and the content of the essential oil decreases. The treatment with Hattrick DP-50 speeds up neither the process before blooming nor the bloom phase, which is important for the harvest of peppermint.

These findings are in agreement with the results of other authors who studied the dependence of main and secondary components of the peppermint essential oil. The content of peppermint characteristic compounds such as menthol, menthyl acetate, and neomenthol increased in the basipetal direction, whereas menthone and isomenthone showed higher levels in the acropetal direction

Table 2. Percentage of the main and some secondary components of the essential oil from the above-ground parts of treated peppermint plants in individual developmental phases

Onthogenesis phase	Parts of the plant	Percentage of the essential oil content	
		untreated	treated
Bifurcation	apical parts of leaves	0.87	0.79
	middle parts of leaves	0.85	0.80
	basal parts of leaves	0.72	0.75
	herba	0.68	0.51
Bud-set	apical parts of leaves	1.86	1.82
	middle parts of leaves	1.69	1.54
	basal parts of leaves	1.43	1.51
	herba	0.85	0.93
Beginning of bloom	apical parts of leaves	2.91	3.35
	middle parts of leaves	2.73	2.64
	basal parts of leaves	2.10	2.27
	herba	1.69	1.39
Full in bloom	apical parts of leaves	3.85	3.92
	middle parts of leaves	3.14	3.65
	basal parts of leaves	2.05	1.94
	herba	1.85	1.86
Fade of bloom	apical parts of leaves	2.47	2.53
	middle parts of leaves	2.01	2.17
	basal parts of leaves	1.25	1.32
	herba	1.17	1.08

(Rohloff 1999). Limonene and menthone are the major monoterpenes present in the youngest leaves. The portion of limonene declines rapidly with development, whereas that of menthone increases in prominence with increasing oil yield and declines only at later stages as menthol becomes the dominant monoterpene constituent (Burbott and Loomis 1969, Croteau and Martinkus 1979). Brunn et al. (1991) and Voirin and Bayet (1996) examined variations in essential oil content within regions of individual leaves and between leaves of various ages on shoots of peppermint. The studies revealed relatively high menthol content in older leaves with an increase in menthyl acetate content during leaf senescence. Piccaglia et al. (1993) observed that the ageing of the crop diminished the biomass yield, plant height and leaf size and produced oils with higher menthofurane and menthyl acetate content.

As for the content of essential oils in specific groups of leaves, the highest proportion was determined in the leaf pairs of the apical parts of leaves, followed by the relatively lower proportions in the middle parts of leaves, with the basal parts of leaves containing the lowest percentage of essential oils. The essential oil content in specific pairs of leaves was increasing during different developmental stages both for the treated and untreated plants and it has been generally higher in the leaves than in the herb (Table 3).

Some authors (Burbott et al. 1983, Voirin et al. 1990, Amelunxen and Intert 1993, Voirin and Bayet 1996, Turner et al. 2000) reported that young leaves contain larger amounts of the essential oil than older, fully developed leaves, on which the secerning forms (glands) and gland trichonomas start to degenerate. Our results are in agreement with these findings.

The effects of soil quality, climate and agrochemical properties and some other factors on the quantity and quality of essential oil of *Mentha* × *piperita* are not so common among the aims of scientific studies (Maksimovic et al. 1999). In

Table 3. Percentage of the essential oil content from some above-ground parts of untreated and treated peppermints plants in individual developmental phases

Onthogenesis phase	Parts of the plant	Percentage of the essential oil content	
		untreated	treated
Bifurcation	apical parts of leaves	0.87	0.79
	middle parts of leaves	0.85	0.80
	basal parts of leaves	0.72	0.75
	herba	0.68	0.51
Bud-set	apical parts of leaves	1.86	1.82
	middle parts of leaves	1.69	1.54
	basal parts of leaves	1.43	1.51
	herba	0.85	0.93
Beginning of bloom	apical parts of leaves	2.91	3.35
	middle parts of leaves	2.73	2.64
	basal parts of leaves	2.10	2.27
	herba	1.69	1.39
Full in bloom	apical parts of leaves	3.85	3.92
	middle parts of leaves	3.14	3.65
	basal parts of leaves	2.05	1.94
	herba	1.85	1.86
Fade of bloom	apical parts of leaves	2.47	2.53
	middle parts of leaves	2.01	2.17
	basal parts of leaves	1.25	1.32
	herba	1.17	1.08

spite of this, Aubert et al. (1998) described the effects of fungicide residues on inhibition of the biosynthesis of sterols and triterpenes influencing the aromatic composition of plants. Many papers focused on various effects of fungicides on plants. Marcucci and Filiti (1984), Pavlik and Jandurova (2000) studied correlations between pollen germination and enzyme activity after fungicide treatment, Jastrzebska and Kucharski (2007) focused on the effects of fungicides on the activity of soil enzymes. Fungicides influence the activity of plant enzymes as well as the activity of enzymes of microorganisms. Recent studies suggest that they can also affect the secondary metabolism of plants to a relatively important extent. The study of the influence of pesticides on the composition of essential oils of medicinal plants can be generally characterized as the influence of stress conditions on the plant's metabolism. The abiotic stress influences the formation and the composition of secondary metabolites, especially the production of phytoalexins (Reilly and Klarman 1972, Cartwright et al. 1980). Both biotic and abiotic stresses can effect plant volatile organic compounds emissions through their strong impact on jasmonic acid levels (Filella et al. 2006). Many plant species defend themselves against herbivorous insects indirectly by producing volatiles in response to herbivory (Van Poecke et al. 2001, Vuorinen et al. 2004).

According to the presented results it is evident that the treatment of peppermint plants with Hattrick DP-50 does not bring about the changes in the content and composition of its essential oil.

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Received on June 26, 2009

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