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INFLUENCE OF SOME PHENYLUREA CYTOKININS ON SPEARMINT ESSENTIAL OIL COMPOSITION

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Summary. The influence of phenylurea cytokinins DROPP and 4PU-30 on essential oil composition (GC analysis) of cineole-type spearmint cultivar CS-87 was studied. Increase of the major components 1,8-cineole (with 13.8–22.5%) and *p*-cymene (with 13–16.5%) and nearly double decrease of carvacrol and thymol was observed. Since these alterations affected insignificantly the essential oil quality we recommend application of 4PU-30 at 25 mg/l and DROPP at 100 mg/l in case the productivity and composition of essential oil are well-balanced and the specific pharmacological activity could be guaranteed.

Key words: spearmint, essential oil composition, 1,8-cineole, phenylurea cytokinins, DROPP, 4PU-30

Abbreviations: DROPP – 50% thidiazuron (TDZ) – (N-phenyl-N´-(1,2,3-thidiazol-5-yl) urea); 4PU-30 – N-(2-chlor-4-pyridyl)-N´-phenylurea

Introduction

Cytokinins produce various physiological effects on plants. In case essential oil plants are treated with such phytoeffectors for the purpose of increasing their herbage and oil productivity it is of great importance to bear in mind the probable alterations of their essential oil composition.

Zlatev et al. (1978, 1980, 1990), Iliev et al. (1983) and Iliev (1991, 1991a) have established an increase of productivity of herbage, essential oil and rhizomes in *Mentha piperita* L. without unfavourable changes in essential oil composition after

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treatment with purine- and urea-type cytokinins. The authors recommend phenylurea-type cytokinins for practical use because, compared to purine-type, they are more active, not expensive and non toxic.

Ivanova and Iliev (1993) observed the stimulative effect of some phenylurea cytokinins (BAP, DPU, TDZ, and 4PU-30) on the rooting process in peppermint *in vitro* propagation. The insignificant influence of TDZ and 4PU-30 on the essential oil composition of *in vitro* produced peppermint plants was established (Ivanova et al., 1996). Stimulative effects of foliar-apllied purine- and phenylurea-type cytokinins on plant growth and essential oil content without alteration of the oil composition were reported for some Lamiaceae species: *Mentha piperita, M. spicata* (with carvone as a main component)*, M. suaveolens, Lavandula vera, Salvia officinalis* (El-Keltawi and Croteau, 1986, 1987). It was established that the primary influence of cytokinins was to stimulate the overal accumulation of monoterpenes typical of the respective essential oil.

Production of *Mentha spicata* (L.) Huds. essential oil is a relatively new line in Bulgarian mint manufacturing. The most widely known spearmint oil contains as a major component carvone. In the 1974–1980 period forms with different main components (linalool, cineole, or mixed) were obtained on the basis of the chemical hybrid variability of this species. Different fragrances of essential oils obtained from these new varieties – Mechta, Lina, Krista, CS-87 and CS-26 – expand the apllication of spearmint in pharmaceutical, food-processing and tobacco industries (Jankulov et al., 1979; Stoeva and Yankulov, 1987; Stoeva, 1990).

The positive effect of the derivatives from the well-known cytokinin DPU (N,N´-2-phenylurea): DROPP – 50% thidiazuron (TDZ) – (N-phenyl-N´-(1,2,3-thidiazol-5-yl) urea) and 4PU-30 – N-(2-chlor-4-pyridyl)-N´-phenylurea, applied to *Mentha piperita*, motivates our experiments with new varieties of *Mentha spicata*. Results concerning carvone spearmint cv. Mechta demonstrated an inscrease of the essential oil productivity (maximum by 56% compared to the control sample after treatment with DROPP 100 mg/l in the spring). Qualitative oil alterations relate only to changes in the proportions of components (in general a drop of carvone with maximum 34.04% was compensated for by a rise of limonene with 32.22%) (Stoeva et al., 1995). The authors have established increase of the essential oil yield up to 50 kg/ha after treatement of cineole spearmint CS-87 with the same plant regulators (unpublished data).

With the present study we aimed to investigate the alterations in oil composition of cineole-type spearmint cultivar CS-87 caused by cytokinins of phenylurea type.

Material and Methods

The agrobiological and economically valuable characteristics of cineole-type *Mentha spicata* (L.) Huds. cv. CS-87 are: yield of air-dried mass 3 350 kg/ha, essential oil

content in air-dried mass 1.25%, yield of essential oil 40 kg/ha, content of the main component 1,8-cineol up to 30% depending on environmental conditions. Essential oil is suitable for pharmacy and as food additive.

The experiments were conducted on the field of the Institute of Botany in 4 replications per parcel area of 1 m^2 by the generally accepted agrotechnical method of row spacing at 70 cm, amount of fertilizers – 300 kg/ha ammonium nitrate and 500 kg/ha superphosphate, moderate watering, no herbicides.

Treatments: var. 1 – Control (water); var. 2 – 4PU-30 – 25 mg/l; var. 3 – 4PU-30 – 50 mg/l; var. 4 – DROPP – 50 mg/l; var. 5 – DROPP – 100 mg/l.

Plants were sprayed with cytokinin solutions in the autumn, 5 weeks after harvesting, in order to increase productivity for the next harvesting year by stimulation of rhizomes' dormant buds.

Above-ground parts were harvested at full flowering stage (August 15th). The essential oil was obtained from air-dried plant material twice of each variant (25 g – average sample from all four replications) by one-hour hydrodistillation with a Clevenger-type apparatus.

The essential oil composition was determined by GC at InPaCo LTD, Plovdiv, by Carlo Erba Vega 6000 (FFAP 25 m×0.32 mm fused silica capillary column, Supelcovax, film thickness 0.45 μm). Temperature programme: 70 °C (min), 70 °–150 °C (6 °C/min), 150 °–230 °C (12 °C/min), 230 °C (5 min); carrier gas Ar at a flow rate 0.82 m^3/min; detector and injector temperature 280 °C.

Results and Discussion

The basic components which characterize the pharmacological activity and fragrance of the investigated essential oil are: 1,8-cineole 26.33%, *p*-cymene 17.92%, carvacrol 17.76%, thymol 6.04% (Tabl. 1).

In all four variants the content of 1,8-cineole increased: from 29.79% (var. 5) to 32.254% (var. 2). Lower concentrations of both phytoeffectors (4PU-30 25 mg/l and DROPP 50 mg/l) proved more effective which is valid for *p*-cymene, too (20.24% at var. 2 and 20.87% at var. 4). On the other hand, the reverse correlation is valid for phenols carvacrol and thymol. They decreased nearly twice in content at all variants and reached lowest values at lower concentration of phytoeffectors: carvacrol 9.76% (var. 2) and 10.33% (var. 4); thymol 3.60% (var. 2) and 3.94% (var. 4).

Most significant increase was established in the minor compounds as menthol (1500.00% compared to the control) and limonene (1100.00%), but since their participation is quite scanty it is insignificant for the oil fragrance. Theoretically, the stimulative effect of phenylurea cytokinins on the synthesis of menthol compounds corresponds to the reported data for *Mentha piperita* where menthol is a basic component (Zlatev et al., 1990).

Table 1. GC analyses of essential oil composition (percentage) in cineole spearmint CS-87

Compound	var.1	var.2		var.3		var.4		var.5	
	Control (water)	4PU-30 (25 mg/l)	% to control	4PU-30 (50 mg/l)	% to control	DROPP (50 mg/l)	% to control	DROPP (100 mg/l)	% to control
α-pinene	4.16	4.69	112.74	3.82	91.83	4.86	116.83	4.80	115.38
β-pinene	1.88	1.91	101.60	1.96	104.26	2.44	129.79	2.31	122.87
sabinene	2.39	2.90	121.34	1.82	76.15	2.37	99.16	2.45	102.51
myrcene	2.09	2.97	142.11	2.26	108.13	2.91	139.23	3.16	151.20
α-terpinene	0.33	0.25	75.76	0.47	142.42	0.64	193.94	0.14	42.42
limonene	0.06			0.66	1100.00	0.34	566.67		
1,8-cineole	26.33	32.25	122.48	30.48	115.76	29.96	113.79	29.79	113.14
ocimene	0.37	0.37	100.00	0.33	89.19	0.39	105.41	0.41	110.81
γ-terpinene	1.12	1.58	141.07	1.20	107.14	1.48	132.14	1.65	147.32
p-cymene	17.92	20.24	112.95	16.69	93.14	20.87	116.46	20.10	112.17
terpinolene	0.05	0.05	100.00	0.04	80.00	0.05	100.00	0.07	140.00
trioctanol	0.16	0.24	150.00	0.18	112.50	0.21	131.25	0.19	118.75
menthone	0.67	1.02	152.24	0.10	14.93	0.68	101.49	0.78	116.42
menthofuran	0.05	0.06	120.00	0.07	140.00	0.03	60.00	0.07	140.00
isomenthone	0.04	0.07	175.00	0.07	175.00	0.06	150.00	0.03	75.00
camphor	0.14	0.14	100.00	0.07	50.00	0.08	57.14	0.12	85.71
linalool	0.09	0.09	100.00	0.34	377.78	0.08	88.89	0.55	611.11
caryophyllene	2.00	2.29	114.50	1.60	80.00	1.73	86.50	2.29	114.50
unidentified				0.47		0.64			
menthol	0.02	0.07	350.00	0.07	350.00	0.05	250.00	0.30	1500.00
pulegon	0.26	0.32	123.08	0.22	84.62	0.21	80.77		
unidentified	1.87	1.97	105.35	1.94	103.74	1.80	96.26	1.89	101.07
neril acetate	1.27	1.67	131.50	1.65	129.92	1.56	122.83	1.59	125.20
carvone	0.72	0.72	100.00	0.40	55.56	0.08	11.11	0.77	106.94
unidentified	5.82	5.27	90.55	7.50	128.87	6.38	109.62	6.39	109.79
thymol	6.04	3.60	59.60	5.27	87.25	3.94	65.23	4.11	68.05
carvacrol	17.76	9.76	54.95	14.11	79.45	10.33	58.16	10.64	59.91

In CS-87 carvone is a minor component (0.72%) and its content decreased at the higher concentration of 4PU-30 (nearly twice) and at the lower concentartion of DROPP (with nearly 90%). Similar carvone alterations were established in cv. Mechta (*Mentha spicata*), where carvon is the main component (Stoeva et al., 1995).

It could be concluded that application of phenylurea cytokinins to cineole-type spearmint CS-87 is favourable concerning increase of herbage and essential oil productivity. We recommend 4PU-30 at 25 mg/l and DROPP in 100 mg/l. At these concentrations composition of essential oil are well-balanced, which should guarantee the specific pharmacological activity of this drug.

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References

El–Keltawi, N. E., R. Croteau, 1986. Influence of ethephon and daminozide on growth and essential oil content of peppermint and sage. Phytochemistry, 25, 1285–1288.

El–Keltawi, N. E., R. Croteau, 1987. Influence of foliar applied cytokinins on growth and essential oil content of several members of the Lamiaceae. Phytochemistry, 26, 891–895.

Iliev, L., S. Zlatev, G. Vasilev, 1983. Cytokinins of purine and non-purine type and productivity of peppermint. In: Proceed. III Intern. Symp. Plant Growth Regulators, Varna 1981. Eds. D. Lilov et al., Publ. House Bulg. Acad. Sci., 776–779.

Iliev, L., 1991. Possibilities for applying cytokinins in plant growing. Agricultural Science, 29 (1–3), 36–43 (in Bulg.).

Iliev, L., 1991. Main physiological properties of cytokinins. Plant Physiology (Sofia), 17, 85–101 (in Bulg.).

Ivanova, K., L. Iliev, 1993. Comparison between BAP and some phenylurea cytokinins during *in vitro* peppermint propagation. Biotechnol. & Biotechnol. Eq., 7, 24–27.

Ivanova, K., M. Manov, L. Iliev, B. Stefanov, 1996. Effect of cytokinins on the essential oil composition of *in vitro* produced peppermint plants. Biotechnol. & Biotechnol. Eq., 10, 44–48.

Jankulov, J., T. Stoeva, I. Ivanov, A. Zuzulova, 1979. Gewinnung von Minzölen mit neuen Duftkomponenten. Compt. rend. Acad. bulg. Sci., 32, 981–983.

Stoeva, T., Y. Yankulov, 1987. Half-sibs generations of *Mentha spicata* Hudson in the breeding new varieties with a cineol flavour. In: Proceed. IV Nat. Conf. Bot., Vol. II, Sofia 1987, 67–74 (in Bulg.).

Stoeva, T., 1990. Variability of semi-sib progenies of *Mentha spicata* (L.) Huds. and possibilities for selection of promising forms. Ph. D. Thesis. Institute of Botany, Publ. House Bulg. Acad. Sci. (in Bulg.).

Stoeva, T., T. Donchev, L. Iliev, 1995. Influence of some phenylurea type cytokinins on the productivity of spearmint cultivar Mechta essential oil. In: Proceed. Iubileum Symposium “100 years of burthday of the Acad. B. Stefanov”, Vol. 1, Sofia 1994, 223–227.

Zlatev, S., L. Iliev, G. Vasilev, M. Zlateva, 1978. Influence of some cytokinins on herb and essential oil yield of peppermint. Horticultural Science (Sofia), 15, 51–56 (in Bulg.).

Zlatev, S., L. Iliev, G. Vasilev, M. Zlateva, 1980. Influence of certain purine and urea cytokinins on rootstock yields of peppermint. Compt. rend. Acad. bulg. Sci., 33, 555–559.

Zlatev, S., T. Donchev, L. Iliev, 1990. Influence of some phenylurea-type cytokinins on productivity of peppermint. Plant Physiology (Sofia), 16, 59–64 (in Bulg.).