Effect of clonal reproduction on quantitative indices and component composition of essential oil of peppermint varieties

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Introduction

According to State Statistics Service data nearly 50 species of plants whose raw materials are used for medicinal preparation manufacture are cultivated in Ukraine. Structural parts of these plants contain biologically active substances which positively influence the human organism. Peppermint is one of the most widespread and traditionally cultivated species. It is cultivated as industrial culture in all climatic zones of Ukraine, Moldova, Russia (Krasnodar territory and North Caucasus), Belarus, and also in many countries of Europe, Central Asia, Africa and the North America. Such soil-climatic variety of cultivation zones testifies to the considerable adaptive potential of this culture. The demand for medicinal preparations on the basis of natural vegetative raw materials has increased in the last 10–15 years and the Ukraine home market does not satisfy the needs of the domestic chemical-pharmaceutical industry, which requires every year nearby 50 tonnes of M. piperita essential oil and 20–25 tonnes of natural menthol (Shelud’ko, 2004). Mint is a source for obtaining pharmaceutic leaf, essential oil and its components, which are widely used in the chemical-pharmaceutical, perfume-cosmetic, confectionery, food, lacquer and spirits, tobacco, paint and coatings industries and medicine (Vojtek, 1999; Tkachenko, 2011).

The biologically active substances of peppermint are complex and its essential oil shows anti-inflammatory, antimicrobial, antifungal, antiviral, anthelmintic, antiparasitic, analgetic, spasmyloytic, cholagogic, mucolytic and bronchoolytic action, normalises central and vegetative nervous system activity, limits rotting and fermentation processes, strengthens motor secretory intestinal tract function, prevents peptic ulcer development, expands coronary vessels, improves heart blood supply, reduces pressure in blood circulation small circle, and also shows...
menthol, pulegone and menthofuran by secondary metabolites biosynthesis induction. It is shown in the research of Slovak scientists that in the essential oil composition of M. piperita breed Kristinka in culture in vitro the menthofuran content increased after addition of 2,4-dichlorophenoxacyetic acid (2,4-D) and CoCl₂ to the nutrient medium and after BAP and zeatin application pulegone content increased (Fejer et al., 2018).

Therefore, the topicality of the given work consists in studying for plants at clonal microreproduction in vitro the influence of the improvement process on the further secondary metabolites synthesis, in particular terpenoids, which are a part of the essential oils of mint. In this connection, the purpose of our work is to investigate within three years the quantitative content and qualitative composition of essential oil components of peppermint breeds of the Ukrainian selection Lebedina Psinya, Lubenchanka, Lidiya, Ukrainska Perechyna, Mama, Chornolista, received from plants after vegetative reproduction and clonal microreproduction in vitro.

Materials and methods

The research objects are the essential oil samples of pepper mint breeds Lebedina Psinya (5), Lubenchanka (7), Lidiya (8), Ukrainska Perechyna (9), Mama (10), Chornolista (11). The breed Lebedina Psinya is cultivated for obtaining pharmaceutical leaves and for essential oil processing. The breed Lubenchanka is cultivated for pharmaceutical industry technical purposes – obtaining essential oil and menthol. The breeds Lidiya, Chornolista, Mama are used for obtaining pharmaceutical leaves (Grodzinskij, 1992; Shetul’ko, 2004).

The research was conducted during 2014-2017 within the framework of the scientific theme “Lamiaceae family essential oil medicinal plants. Biotechnological reproduction foundations for receiving high-quality planting material” (number of the state registration 01160.001904).

Methods of isolated tissues and organs culture in vitro and chemotherapy were used for pepper mint improvement in 2014. Murashige and Skoog (Murashige & Skoog, 1962) nutrient medium, in which 0.75 mg/l BAP, 0.1 mg/l adenine, 0.05 mg/l indolebutyric acid (IAA) and 0.5 mg/l gibberellic acid (GA), and also vincide Ribavirin (1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide, “Sigma-Aldrich”, USA) in 10 mg/l concentration were added, paravirization was used for above-mentioned peppermint breeds of Ukrainian selection explants introduction and microreproduction (Tulan’kova-Sereda et al., 2016).

The experiment was conducted four times in Lubensky district of Poltava region in the territory of the Research Station of Medicinal Plants of the Institute of Agroecology and Nature Management of NAAS of Ukraine annually since 2015 in field conditions, in which the influence of clonal microreproduction and chemotherapy was investigated on the quantitative indices and componental composition of essential oil of pepper mint breeds Lebedina Psinya, Lubenchanka, Lidiya, Ukrainska Perechyna, Mama, Chornolista. Vegetatively reproduced planting material of the mint breeds was used as the control variants. Mint sprots, which were used for trial establishment, met the requirements of the standard document “Mint Sprouts” (Technic Specifications 10-04-13-48-88 “Mint Sprouts. Technic Specifications”).

The research was conducted according to Moloczkij et al. (2006), Esbenko et al. (2014), Shetul’ko & Kucenko (2013) techniques. Pepper mint raw materials for obtaining essential oil were selected annually with time requirements compliance, which is defined in technology regulations for the investigated kind. In particular, the investigated breeds overground part was cut in the mass flowering phase as numerous researches specify a tendency to increase in essential oil content during this period (Tanasienko, 1985; Kirichenko, 2008; Morozov & Hazieva, 2013). Raw materials from plants after vegetative reproduction and plants to which clonal microreproduction in vitro was applied were selected on each of plots with the use of the linear metre method.

Essential oil was received by steam distillation by A. S. Ginzberg’s technique (Heffendel et al., 1967) with the subsequent recalculaton on dry weight. For this purpose, 50 g milled raw materials were placed in wide neck flask with 3,000 ml capacity, then 1,500 ml water was added and it was closed with a rubber stopper with a ball check refrigerator. The calibrated receiver was suspended on metal hooks on the stopper.
that plants of this breed in culture in vitro had the maximum rate of reproduction 1:15 for one passage. Essential oil quantity in vegetatively reproduced plants of breed Chornolista was 3.77%, and in plants after culture in vitro it increased to 3.83%. It is necessary to note that the breed Chornolista is recognised by the State Commission of Strain Test in Ukraine as a peppermint meeting the state standard for the purpose of obtaining pharmaceutical leaves and manufacturing phytoconstitutions.

Essential oil componental composition and its separate fractions proportion are defined for peppermint breeds Lebedina Pisnya, Lubenchanka, Lidiya, Ukrainska Perechna, Mama and Chornolista. Limonene, cineole, menthone, menthofuran, isomenthone, menthyl acetate, \( \beta \)-caryophyllene, isomenthol, menthol, pulegone, germacrene, pipertone, carvone were identified among compounds which are components of peppermint essential oil (Table 2, 3).

### Table 2

| Essential oil component | European Pharmacopoeia standard | Peppermint breeds | Peppermint breeds | Peppermint breeds | Peppermint breeds |
|-------------------------|---------------------------------|-------------------|-------------------|-------------------|-------------------|
|                         |                                 | Lebedina Pisnya   | Lubenchanka       | Lidiya            | Ukrainska Perechna |
| Limonene                | 1.0–5.0                         | 3.2               | 3.5               | 1.3               | 0.9               | 3.2               | 1.1               |
| Cineole                 | 3.05–14.0                      | 0.2               | 0.3               | 0.1               | 2.8               | 0.2               | 3.8               |
| Menthone                | 14.0–32.0                      | 14.9              | 9.3               | 12.6              | 18.8              | 12.6              | 23.5              |
| Menthofuran             | 1.0–9.0                        | 0.5               | 4.4               | 0.0               | 9.3               | 0.5               | 4.8               |
| Isomenthone             | 1.5–10.0                       | 2.8               | 2.5               | 17.6              | 5.3               | 2.7               | 4.0               |
| Menthyl acetate         | 2.8–10.0                       | 2.4               | 3.3               | 2.9               | 8.5               | 2.6               | 3.1               |
| Menthol                 | 30.0–55.0                      | 70.1              | 68.0              | 60.5              | 38.4              | 72.0              | 30.7              |
| Isomenthyl             | 3.0–11.0                       | 2.0               | 3.1               | 1.1               | 2.5               | 2.0               | 6.5               |
| Pulegone                | Up to 4.0                      | 0.0               | 0.0               | 0.0               | 6.1               | 0.0               | 11.1              |
| Carvone                 | Up to 1.0                      | 0.0               | 0.1               | 0.2               | 0.1               | 0.0               | 0.6               |
| Minor compounds         | 3.8–5.5                        | 5.5               | 3.9               | 3.7               | 4.2               | 7.4               | 11.0              |

### Table 3

| Essential oil component | European Pharmacopoeia standard | Peppermint breeds | Peppermint breeds | Peppermint breeds | Peppermint breeds |
|-------------------------|---------------------------------|-------------------|-------------------|-------------------|-------------------|
|                         |                                 | Lebedina Pisnya   | Lubenchanka       | Lidiya            | Ukrainska Perechna |
| Limonene                | 1.0–5.0                         | 2.9               | 3.5               | 1.5               | 0.7               | 1.9               | 1.0               |
| Cineole                 | 3.1–14.0                       | 0.2               | 0.3               | 0.1               | 3.3               | 0.5               | 3.1               |
| Menthone                | 14.0–32.0                      | 8.8               | 8.9               | 11.0              | 20.1              | 12.9              | 21.8              |
| Menthofuran             | 1.0–9.0                        | 0.3               | 3.8               | 0.2               | 6.8               | 0.3               | 4.5               |
| Isomenthone             | 1.5–10.0                       | 2.8               | 2.8               | 16.8              | 6.8               | 1.5               | 3.3               |
| Menthyl acetate         | 2.8–10.0                       | 3.7               | 3.1               | 2.8               | 5.8               | 1.0               | 2.9               |
| Menthol                 | 30.0–55.0                      | 74.1              | 68.8              | 61.4              | 44.2              | 72.6              | 33.3              |
| Isomenthyl             | 2.0–8.2                        | 2.8               | 1.2               | 2.5               | 43.6              | 6.4               |
| Pulegone                | Up to 4.0                      | 0.0               | 0.0               | 0.0               | 3.1               | 0.0               | 11.9              |
| Carvone                 | Up to 1.0                      | 0.0               | 0.2               | 0.2               | 0.1               | 0.2               | 0.9               |
| Minor compounds         | 5.1–5.8                        | 5.8               | 4.8               | 6.7               | 4.8               | 6.7               | 11.0              |

Breeds Lebedina Pisnya – 70.1–74.1% (Fig. 1), Lubenchanka – 68.0–68.8%, Lidiya – 60.5–61.4% and Mama – 72.0–72.6% contain the maximum menthol content as the basic marking essential oil component according to the received data.

Essential oil contained almost half the abovementioned content of menthol, 38.4–44.2% and 30.7–33.3%, respectively in vegetatively reproduced culture in vitro breeds Ukrainska Perechna and Chornolista plants, which correlates with the high menthone content in these samples. The proportion of menthone was 18.8% in the essential oil of plants of the breed Ukrainska Perechna without culture in vitro application, after clonal microproduction – 20.1%, and in the breed Chornolista...

### Results

The highest indices of essential oil content among investigated pepper mint breeds of Ukrainian selection were noted for Lubenchanka, Mama and Lebedina Pisnya – 4.02%, 3.98% and 3.84% respectively (Table 1). These indices are especially important for breeds Lubenchanka and Lebedina Pisnya, which are cultivated for obtaining essential oil as the basic product.

#### Table 1

| Peppermint breeds | Variants | 2015       | 2016       | 2017       |
|-------------------|----------|------------|------------|------------|
| Lebedina Pisnya   | VR       | 3.79 ± 0.010 | 3.82 ± 0.013 | 3.82 ± 0.006 |
| in vitro          | VR       | 3.83 ± 0.013 | 3.85 ± 0.008 | 3.85 ± 0.008 |
| Lubenchanka       | VR       | 3.98 ± 0.008 | 4.01 ± 0.014 | 3.99 ± 0.010 |
| in vitro          | VR       | 4.00 ± 0.013 | 4.03 ± 0.017 | 4.02 ± 0.015 |
| Lidiya            | VR       | 3.20 ± 0.013 | 3.24 ± 0.016 | 3.23 ± 0.013 |
| in vitro          | VR       | 3.33 ± 0.010 | 3.35 ± 0.008 | 3.34 ± 0.017 |
| Ukrainska         | VR       | 3.63 ± 0.014 | 3.67 ± 0.013 | 3.63 ± 0.015 |
| Perechna          | VR       | 3.66 ± 0.010 | 3.71 ± 0.014 | 3.70 ± 0.010 |
| in vitro          | VR       | 3.90 ± 0.013 | 3.94 ± 0.008 | 3.91 ± 0.010 |
| Mama              | VR       | 3.95 ± 0.010 | 3.99 ± 0.010 | 3.99 ± 0.008 |
| in vitro          | VR       | 3.74 ± 0.014 | 3.78 ± 0.010 | 3.77 ± 0.013 |
| Chornolista       | VR       | 3.81 ± 0.016 | 3.86 ± 0.013 | 3.83 ± 0.010 |
| in vitro          | VR       | 3.38 ± 0.010 | 3.83 ± 0.010 | 3.83 ± 0.010 |

Notes: VR – vegetatively reproduced plants; in vitro – plants treated in culture in vitro; * – differences are statistically significant at \( P < 0.05 \), ** – at \( P < 0.01 \) comparing with the control.

The Lidiya breed of vegetative reproduced plants contained the least essential oil quantity 3.22%, however this index increased by 0.12% in plants after culture in vitro and was 3.34%. Essential oil quantity in vegetatively reproduced plants of the breed Ukrainska Perechna after culture in vitro was accordingly 3.64% and 3.69%. It is important also,
lista – 23.5–21.8%, but total menthol and menthone content in these plants was high enough and was in the breed Ukrainska Perechna – 57.2–64.3%, Chornolista – 54.1–55.0%. Total menthol and menthone content in high menthol breeds was considerably higher, in particular in Lebedina Pisnya – 85.0–82.9%, Lubenchanka – 77.3–77.7%, Lidiya – 73.1–72.3% and Mama – 85.5–82.5%.

Menthone quantity decreased in essential oil of four breeds of vegetatively reproduced plants and after culture in vitro, in particular in the pepper mint breed Lebedina Pisnya by 6.15%. In the breeds Ukrainska Perechna and Mama it slightly increased. The quantity of such major essential oil components as menthol and menthone in different breeds fluctuated.

Limonene concentration in all essential oil samples was within the range of the recommended European pharmacopoeia (Ph. Eur.) values, under which requirements this non-toxic monocyclic terpene representative should be in the range of 1.0–5.0%. Only in both essential oils of plant samples of the breed Ukrainska Perechna was it was lower than the European Pharmacopoeia standard by 0.1–0.4% (Ph. Eur. 8, 2014). The insignificant reduction in this essential oil component was observed in all pepper mint plants breeds after culture in vitro application by 0.1–1.3%, except for the breed Lidiya in which the proportion of limonene increased by 0.3%.

Analysis of the amount of cineole contained in the essential oil samples showed that for the majority of breeds this component amounted to less than 1%, except for two breeds – Ukrainska Perechna and Chornolista, but its quantity nevertheless is below European pharmacopoeia requirements. Only the essential oil which was received from vegetatively reproduced plants of the breed Chornolista corresponded to the European pharmacopoeia standards (3.5–14.0%) (Ph. Eur. 8, 2014).

It is necessary to notice that essential oil received by steam distillation by A. S. Ginzberg’s technique can yield underestimated results on more volatile components, which include limonene and cineole, in comparison with the technique which is used in the European pharmacopoeia.

An insignificant excess of menthofuran content over the established standard European pharmacopoeia requirements was noted only in the breed Ukrainska Perechna among investigated essential oil samples. Menthofuran content did not exceed 1% in essential oil breeds Lebedina Pisnya, Lidiya, Mama. The breeds Lubenchanka and Chornolista indices correspond to Ph. Eur. (1.0–9.0%). The general tendency to menthofuran reduction on average by 0.2–2.5% among essential oil peppermint plants samples, which were treated in culture in vitro, except for the breed Lidiya, was observed. In the essential oil of vegetatively reproduced plants this component was not revealed by us, and in plants-regenerants raw materials its share was 0.2%. It is known, that mint flowers contain a significant menthone and menthofuran amount (Tanasienko, 1985). Menthofuran content of less then 9% does not worsen essential
oil quality, but usually it is accompanied by other substances with an unpleasant smell.

Essential oil of the uncloned breed Ukrainska Perechna had increased pulegone content – 6.1%, at the same time in this breed essential oil of plants after culture in vitro content of this component decreased by 50%, which corresponds to the European pharmacopoeia requirements. In essential oil samples of vegetatively reproduced plants of the breed Chornolista, pulegone amounted to 11.1% and after culture in vitro application its content increased to 11.9%, but such quantity exceeds European pharmacopoeia standards by three times (to 4%). These breeds were created in Ukraine as English peppermint analogues, and pulegone presence is a characteristic feature for them, and its content, according to our data, considerably varies depending on weather conditions, collecting period of raw materials and other factors. Essential oil of peppermint of samples of other breeds of Ukrainian selection, Lebedina Pistrya, Lubenchanka, Lidiya and Mama did not contain pulegone at all, which in excessive quantity (more than 4%) introduces an appreciable shade of standard English peppermint aroma.

All pepper mint essential oil plants of Ukrainian selection samples correspond to European pharmacopoeia standards for content of the terpenoids carvone and piperitone, and in the essential oil of pepper mint breed Mama, to which culture in vitro was not applied, and of the pepper mint breed Lebedina Pistrya (Fig. 1), carvone, which can give unpleasant caraway aroma to the essential oil and which under European pharmacopoeia recommendations should have a concentration of no more than 1%, was completely absent. The low carvone content is a characteristic feature for all pepper mint breeds investigated by us.

Cineole/limonene proportion is an important peppermint essential oil quality index. Two breeds among the investigated essential oil samples correspond to European pharmacopoeia requirements. For Ukraina Perechna without culture in vitro application this index is 2.9 and after culture in vitro application - 5.0, and also in breed Chornolista 3.5 – in uncloned plants and 3.0 – in plants after culture in vitro. According to Ph. Eur. this proportion should be more than 2.0.

If total marking of mint essential oil compounds content will be counted in all essential oil samples, specifically menthone, isomenthone, menthol and isomenthol, it is possible to arrange breeds in such sequence as: Lidiya – 90.3–91.7%; Mama – 89.3–90.3%; Lebedina Pistrya – 87.7–89.8%; Lubenchanka – 82.9–83.2%; Chornolista – 61.3–64.5%; Ukrainska Perechna – 61.6–64.9%.

Main components analysis of qualitative composition and isoprenoïd content in pepper mint breeds Lebedina Pistrya (5), Lubenchanka (7), Lidiya (8), Ukraina Perechna (9), Mama (10), Chornolista (11) leaves established that the first main components axis (F1) was 58.4% from the general dispersion in indices complex, from which the greatest value was for menthol, pulegone, cineole. A slightly smaller contribution to the general dispersion in indices complex, from which the greatest value was for menthol, pulegone, cineole. The first main components axis (F1) was 58.4% from the general dispersion.

| Essential oil indices | F1 (%) | F2 (%) | F3 (%) |
|-----------------------|-------|-------|-------|
| Limonene              | 0.589 | 0.261 | 0.054 |
| Cineole               | 0.889 | 0.003 | 0.058 |
| Menthone              | 0.826 | 0.006 | 0.001 |
| Mentholiferan         | 0.453 | 0.037 | 0.455 |
| Isomenthone           | 0.004 | 0.598 | 0.329 |
| Menthol acetate       | 0.141 | 0.315 | 0.435 |
| B-caryophyllene       | 0.302 | 0.226 | 0.044 |
| Isomenthol            | 0.569 | 0.370 | 0.006 |
| Menthol               | 0.944 | 0.036 | 0.002 |
| Pulegone               | 0.906 | 0.033 | 0.001 |
| Germacruene           | 0.678 | 0.012 | 0.003 |
| Piperitone             | 0.392 | 0.247 | 0.261 |
| Carvone               | 0.602 | 0.131 | 0.182 |
| Minor compounds        | 0.852 | 0.005 | 0.003 |

Notes: * – the greatest contribution to main components weighting.

Axis F1 separates cloned and not cloned in vitro breeds Ukraina Perechna and Chornolista from Lebedina Pistrya, Lubenchanka, Lidiya and Mama. The last differ by absence of pulegone in the leaves and rather high menthol content, which is the key vegetative raw materials quality feature. For breeds Ukraina Perechna and Chornolista, the rather high menthone content accumulation, on the contrary, is characteristic. The second main components axis (F2) was 16.6% from the general dispersion in which the greatest value was for isomenthone. In the investigated breeds group second components axis separates breeds Lidiya and Ukraina Perechna. Axis F2 is 25% from the general dispersion. Mentholiferan and menthyl acetate have the greatest value by the third component. The total 1 and 2 main components dispersion is 75% (Fig. 2). Biplot analysis use allows us to show a number of indices which characterise the investigated breeds by essential oil componental composition specificity.

![Fig. 2](https://via.placeholder.com/150)

**Fig. 2.** Vegetatively reproduced plants (a), and after clonal microreproduction (b) peppermint breeds Lebedina Pistrya (5), Lubenchanka (7), Lidiya (8), Ukraina Perechna (9), Mama (10), Chornolista (11) biplot analysis.

**Discussion**

Essential oil composition is genetically caused. However the proportion of its components depends on many factors, in particular the plant's age, ontogenesis stage, agroprocessing methods, climatic conditions, plants pathogenic organisms and viruses contamination etc. Besides, growth regulators application during the clonal microreproduction in vitro, in turn, is capable of changing the intensity and character of plants' tissues metabolism, but does not cause considerable qualitative differences in these plants landed in vitro essential oil composition, which can be caused by specific fermental systems work stability and genes expression which are responsible for terpenoid synthesis regulation.

It is established by us that yield of essential oil increases in improved plants after methods of tissues and organs culture, chemotherapy in vitro and clonal microreproduction application. Improvement of the plants in culture in vitro can be considered as the reason for this increase in yield. As a clonal microreproduction result, better planting material was received, with better sprout tillering, their leaf cover and accordingly considerable infloroses quantity, and, as is known (Oceana et al., 2015), intensive second level sprouts development and their dense flowering lead to increase in content of essential oil ketones (Tanasieko, 1985). From Fejer et al. (2018) article it is known that 0.5 mg/l cytokinin BAP addition increases sprouts quantity de novo.

Our research found that the quantity of menthol increased in all plants after culture in vitro application, which concurs with the findings presented in Santoro et al. (2013) publication, which studied growth regulators' influence on Mentha piperita plants in vitro aiming at growth and essential oil synthesis maximalisation in micropropagated plants-regenerants. Base nutrient medium was supplemented with 4-indolil-3-
acetic acid (IAA) and BAP separately and in combination in our experiment. Only BAP in 0.6 mg/l concentration addition had led to the highest roots length, their dry weight, sprouts length and internode number, leaves and branching values. Only 0.6 mg/l IAA or simultaneously IAA and 0.6 mg/l BAP processing led to increase in sprouts weights by 50%.

Secondary metabolites synthesis increased only in the case of BAP addition, which resulted in 40% increase in the general yield of essential oil and the basic components (menthone, menthol, pulegone and menthofuran).

Fluctuations in menthol and menthone quantity of different breeds of peppermint were caused by plant genotype, and also connected with continuous synthesis processes, their biochemical transformation and evaporation. Menthol synthesis can occur by pulegone or piperitone restoration, and also in the course of menthol oxidation. Thus, the rather high accumulation of menthone content in the breeds Ukrainska Perechna and Chornolista specifies that for these two breeds there is high activity of fermental systems, which are involved in menthone synthesis pulegone ways.

We have confirmed the precise tendency of menthol to increase by 0.6-5.8% in essential oil of peppermint plants to which we applied culture in vitro, which corresponds to the research of Paric et al. (2017) which has revealed secondary metabolites production stimulation as a result of BAP in concentration from 0.1 to 4.0 mg/l and 0.1 mg/l indole-3-butyric acid (IBA) separately and in combination application, which underlines the great value of the optimum growth regulator and its concentration choice. Also, we have verified that their application led to increase in sprouts and roots quantity.

In the publication of Ivanova et al. (1996) it is shown that menthol and menthone production reduces in plants after culture in vitro, to which cytokinins from purine and phenyl carbamide preparations were applied, but they led to increase in the amount of pulegone and menthofuran, and the highest pulegone content was observed after BAP application. In our research an insignificant increase in pulegone content was characteristic only for the breed Chornolista. Other investigated peppermint breeds before and after improvement did not contain pulegone, which testifies to the absence of this component in the biochemical essential oil synthesis chain of peppermint plants of Ukrainian selection – most likely this is caused by their genotype.

Also, in an article by Slovak scientists culture of M. piperita breed Kristinka in vitro is described, where they had cultivated node segments on nutrient medium and showed that after BAP and zeatin addition in the nutrient medium, the pulegone content increased, and after 2.4-D and CoCl₂ application, menthofuran content in the essential oil composition increased. Our research found that menthofuran was present at 0.2% in the essential oil of cloned plants of the peppermint mint breed Lidiya, though in vegetatively reproduced plants this component was absent. In all other investigated breeds at the same time a general tendency was observed to reduction in menthofuran content on the average by 0.2–2.5%.

Thus, it is established that two components – menthol and menthone are the predominant components of the essential oil of plants of the breeds Lebedina Pisnya, Lubenchanka and Mama, and their total content was: Lebedina Pisnya – 82.9–85.0%, Lubenchanka – 77.3–77.7%, Mama – 82.5–84.6%. In the essential oil of plants of the breed Lidiya three components – menthol, menthone, isomenthone – prevailed, and their total content is 89.1–90.6%; in the breed Ukrainska Perechna five compounds – menthone, menthy1 acetate, menthofuran, menthol, pulegone – prevailed, and their total content is 68.9–81.1%; in the breed Chornolista four compounds – menthone, menthol, isomenthone and pulegone – prevailed, and their total content is 69.9–70.1% from the total essential oil indices sum.

Menthol, menthy1 acetate, cineole and menthone should be present in peppermint leaves (Peppermint leaf) according to the European pharmacopoeia. Also, isomenthone, pulegone and carvone can be determined on a chromatogram. Thymol presence is not required. The optimum for peper mint essential oil components (Peppermint oil) has to be in the proportion – limonene from 1.0 to 5.0%, cineole 3.5–14.0%, menthone 14.0–32.0%, menthofuran 1.0–9.0%, isomenthone 1.5–10.0%, menthyl acetate 2.8–10.0%, menthol 30.0–55.0%, pulegone maximum of 4.0%, carvone no more than 1.0%, isopulegol no more than 0.2% according to European pharmacopoeia standards (Ph. Eur. 8, 2014).

The breed Lidiya was the most productive among the investigated breeds in 2015–2017, its indices were at 2,820 kg/ha level after improvement in vitro and 2,620 kg/ha in the control group. Increase in raw materials productivity after improvement was minimal for the breed Lidiya among investigated breeds and was 7.6%. This can be explained by the fact that culture in vitro and chemotherapy promote mainly the plants’ improvement, protecting their tissues from pathogenic and potentially pathogenic microorganisms, endophytic and epiphytic mushrooms and viruses. Besides, according to existing data, pepper mint breed Lidiya plants have complex resistance to illnesses, which was confirmed in Mishchenko et al. (2015, 2016) publications concerning this breed’s resistance to powdery mildew, rust, anthracnose and to virus diseases.

Differences in productivity indices in breeds Chornolista and Lebedina Pisnya between variants are essential and accordingly are 51.4% and 37.1%. Owing to improvement, culture productivity considerably increased and was 2,120 kg/ha for breed Lebedina Pisnya and 2,550 kg/ha for breed Chornolista. The last is one of the oldest peppermint breeds cultivated in Ukraine, which was created over thirty years ago and for a long time was cultivated in manufacture conditions. The breed Ukrainska Perechna increased productivity by 34.9%, and breed Mama by 26.6%, and this was 1,880 and 2,380 kg/h respectively.

Productivity indices analysis by air-dry foliage weight and pepper mint essential oil quantity determined that in plants after culture in vitro, in comparison with vegetatively reproduced plants, the essential oil quantity per hectare considerably increased in the breed Chornolista by 54.2%, Lebedina Pisnya – by 38.2%, Ukrainska Perechna – by 36.7%, Mama – by 28.5%, Lubenchanka – by 17.1%, Lidiya – by 11.6%.

Thus, the characteristic features of the investigated peppermint breeds of Ukrainian selection are the high menthol content, low carvone, piperitone, pulegone (except breeds Chornolista, Ukrainska Perechna) and menthofuran (except breeds Chornolista, Ukrainska Perechna and Lubenchanka) content. It is necessary to specify, that in the essential oil samples of the breeds Lebedina Pisnya, Lubenchanka, Lidiya and Mama pulegone was completely absent, and in essential oil samples of vegetatively reproduced plants of the breed Mama and both samples from the breed Lebedina Pisnya carvone was absent, which is caused by breed-specific genotypic features and action of fermentative systems, which are connected with given components synthesis.

Conclusions

The qualitative composition of essential oil of vegetatively reproduced peppermint plants raw materials and plants after culture in vitro essentially does not differ and it is within the measures which are characteristic for their genotypes. All raw material samples of improved peppermint plants had increased essential oil quantity. The maximum essential oil content exceeding 4% was received from vegetative raw materials of the breed Lubenchanka. The componental composition of essential oil of mint which was received from vegetatively reproduced plants and plants after culture in vitro varies depending on the breed. In improved in vitro plants a clear tendency to increase in menthol and menthone content is revealed.

A high content of menthol (except for Chornolista, Ukrainska Perechna breeds), and low content of menthofuran (except for Chornolista, Ukrainska Perechna and Lubenchanka breeds) and of carvone, piperitone, pulegone (except the breeds Chornolista and Ukrainska Perechna) are characteristic for the investigated breeds of Ukrainian selection pepper mint. Pulegone was not detected in essential oil samples of the Lebedina Pisnya, Lidiya and Mama breeds. In the Lebedina Pisnya, Lubenchanka and Mama breeds the dominant components which differentiate them in the group of investigated breeds, are limonene, piperitone, menthol, for the Ukrainska Perechna and Chornolista breeds – pulegone, cineole and menthone; for Lidiya breed – iso-menthone.

As a result of clonal microproduction in culture in vitro of pepper mint plants and improvements by viroide Ribavirin (1β-D-ribofurano- syl-1,2,4-triazole-3-carboxamide, “Sigma-Alrich”, USA), the essential oil, in comparison with vegetatively reproduced plants, increased per...
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