Ecological aspects of regulation of the plasticity of accumulation of terpene compounds in plants

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Abstract. Field mint (Mentha arvensis L.) of the Mentha (Lamiaceae) is one of the most important species among medicinal and essential oil-bearing plant. The content of essential oils in the plants of field mint M. arvensis was studied depending on the growing conditions in three biocoenoses, taking into account the increase in soil moisture availability. The output of essential oil from the aboveground part of mint plants was maximum during the mass flowering phase and amounted to 2.7±0.08% in mesohydrophytic biocoenoses, 2.3±0.09% in mesophytic and 1.3±0.11% in xeromesophytic biocoenoses. Cyclic terpenes and terpenoids (65.2 and 59.8%, respectively) predominated in the composition of the field mint plants oil of mesohydrophytic and mesophytic biocoenoses, the soil moisture content of which is more consistent with the mesophilic ancestral ecology of the species, while acyclic compounds accounted for 31.2 and 34.6 %. Simpler acyclic terpenes and terpenoids dominated in the field mint oil profile of xeromesophytic biocoenoses (55.3%). The essential oil of mint plants of all three ecological biocoenoses contained terpenes with a pronounced protective function - γ-terpinene, α-terpinolen, trans and cis-ocimene, eucalyptol (1,8 cineol), which exhibit broad antimicrobial and insecticidal properties. The concentration of these compounds was maximal in plants of the xeromesophytic biocoenoses. While the maximum content of volatile terpenes (β-myrcene, cis-geraniol, nerol acetate), which perform an attractive function (ensures the relationship of plants with insects), is recorded in the mesohydrophytic biocoenoses. The adaptation of mint plants to different ecological conditions is ensured by the shift in the activity of secondary metabolism toward increasing the synthesis of simpler in structure and less energy-intensive components, which provides the population with a selective advantage as the species moves to new habitats that differ in ecological conditions from those specific to ancestral forms.

1. Introduction

The composition plasticity of substances of plant specialized metabolism is associated with the ease of their chemical modification during metabolism. In turn, the ease of chemical modification is due to the ecological and taxonomic control over the biosynthesis of these substances [1, 2, 3]. When growing conditions change, plants trigger several adaptive mechanisms [4, 5]. A significant role in the adaptation of plants to changing environmental conditions is played by substances of specialized metabolism and, in particular, terpene compounds that are part of the essential oils of plants. That is, plants growing in conditions of significant ecological gradients may differ in a specific set of...
secondary compounds in different parts of these gradients. Population analysis, based on the study of terpene compounds content of phenolic and non-phenolic types, allows us to approach the understanding and evaluation of the adaptive norm and to identify some biochemical aspects of the adaptive strategy of populations.

The features of terpene compounds accumulation depending on the growth conditions were analyzed on the example of field mint (*Mentha arvensis* L.) of the *Mentha* genus (Lamiaceae). *M. arvensis* is a perennial branching plant with a recumbent to half its length (from 15 to 20 cm) or upright ascending stem, slightly pubescent, with almost no anthocyanin colour. Its leaves are elliptical with a very weak pubescent upper side on short petioles, the upper leaves are sessile, with saw-toothed edges, a pointed tip and a wedge-shaped base (there is no anthocyanin colour, the green colour of medium degree). The flowers are lilac-pink colour (lilac) collected in multi-flowered spherical false whorls in the axils of the upper leaves, on hairy bare pedicels; calyx bell-shaped, not densely pubescent with triangular short sharp teeth; the corolla is wide-tubular, pubescent with sparse recumbent hairs, lilac; the stamens are longer than the corolla tube or much shorter (underdeveloped).

The species is one of the most important species among medicinal and essential oil plants. In our country, it is widespread throughout the European part, the Pre-Caucasian region, Siberia, and all regions of Central Russia as a common plant [6]. Field mint is able to grow in a wide variety of habitats, preferring sufficiently moist places. It settles in meadows, along the banks of reservoirs, rivers, lakes, along the edge of swamps and in swamp shady forests [7]. The study of the biochemical diversity of *M. arvensis* from different cenosis makes it possible to assess the adaptation processes of plants to different environmental conditions and allows us to assess the adaptive potential of this species.

In this regard, it seemed necessary to study the content and component composition of the essential oil of field mint plants, depending on the growth conditions. The variety of cenosis in which samples of field mint were collected made it possible to make an ecological-coenotic series taking into account the increase in soil moisture availability. The beginning of the series occupied habitats with maximum moisture availability and more consistent with the ecology of the species, and then there were the habitats, the moisture availability of which was more consistent with the modern ecology of the species.

2. Materials and methods

*M. arvensis* plants were collected in the phase of mass flowering in the Moscow region, Lotoshinsky district, in the vicinity of the village of Savostino in three cenopopulations in the floodplain of the Russa River (56°21´53´´ N, 35°42´45´´ E). The collected samples were dried in bundles suspended in air at normal temperature in a shaded area. Essential oil from air-dry material was obtained by hydrodistillation method.

The component composition of the essential oil was determined in the Shared-Access Equipment Centre "Industrial Biotechnology" of the Russian Academy of Sciences (RFMEFI62114X0002) on a Shimadzu GS 2010 gas chromatograph with a GCMS-QP 2010 mass detector; non-polar column SPB-1 (firmly bound methylsilicone) length 30 m, diameter 0.25 mm. The samples were dissolved in benzene in a ratio of 1 : 150 and chromatographed in the temperature gradient mode at the following operating parameters: the temperature of the injector 180°C, the interface 205°C, the detector 200°C. The carrier gas is helium.

The flow through the column is 1 cm³ / min, the flow division is 1: 10. Parameters of the mass detector: registration mode - TIC, mass range 30-400 m/z. The temperature regime for substances with a retention index of lower than 1300 is the thermostat 60°C 3 minutes, then 2°C/min to 230°C and the isotherm is 2 minutes. The temperature regime for substances with a retention index of more than 1300 – the thermostat is 60°C 1 minute, then 4°C/min to 230°C and the isotherm is 2 minutes. To determine the linear retention indices, a mixture of linear paraffins was chromatographed: nonane, monodecane, tridecane, pentadecane, and heptadecane.
3. Results and Discussion

The study of the field mint coenopolulations in the floodplain of rivers located close to each other, and at the same time in different conditions of soil moisture supply due to the natural lowering of the relief, allows us to identify some trends in the terpene compounds accumulation, depending on the growth conditions. The coenopolulations community occupying an elevated habitat belongs to the upland-motley-grass meadow community, and the lowerings – to the hygrophytic-motley-grass community. The features of the terpene complex in the limit of the ecological-coenotic series were considered as a quantitative feature reflecting the content of the leading components, and as a qualitative one, manifested in the ratio of the main components, which is usually characteristic of a certain type of coenosis.

It is known from the literature data and previous studies that the content of essential oil in the aboveground part of plants varies at different stages of seasonal plant development [8]. This is due to the fact that essential oil is generated and stored in the secretory glands of mint plants, which are formed in the early stages of ontogenesis on all organs of the aboveground part of plants (stems, leaves, calyx, corolla). Mint plants are characterized by 2 types of secretory formations: secretory hairs and sessile 10-cell glands. The main fund of the essential oil produced by the plant is concentrated in the secretory glands (trichomes). Their greatest density is observed on leaves aged from 12 to 20 days, and their maximum number is located in the basal part of the leaf, on the middle and apical parts their number is less. A higher density of trichomes is found on the lower part of the leaves, it increases from the leaves of the lower layer to the leaves of the upper one [9, 10].

To study the seasonal dynamics of the essential oil content in field mint plants of different biocoenoses, samples of plant material collected at different stages of ontogenesis were analyzed. The results are presented in Table 1.

| Stage of development          | Content of essential oils, % |
|-------------------------------|------------------------------|
|                               | Mesohygrophytes | Mesophytes | Xeromesophytes |
| Stage of vegetation development | 1.74 ±0.05     | 1.60±0.08 | 0.62±0.10 |
| Stage of flowering            | 2.67±0.08      | 2.31±0.09 | 1.31±0.11 |
| Stage of fructification       | 1.58±0.11      | 1.32±0.15 | 0.69±0.10 |

The results of determining the content of essential oil allowed us to note the following pattern: the maximum yield of essential oil from the aboveground part of mint plants of three ecological biocoenoses was recorded in the phase of mass flowering. In addition, the content of essential oil in the mesohydrophytic biocoenoses was the maximum (2.67%). In the mesophytic biocoenoses, the content decreased slightly (to 2.3%), and in the xeromesophilic biocoenoses (the steppe meadow), it has fallen by 2 times and amounted to 1.3%. During the vegetative and fruiting phases, the essential oil content in plants was lower than in the mass flowering phase by 31-53% and 41-47%, respectively.

Using only a quantitative trait is less informative, although it allows us to identify some trends in their accumulation in connection with the ecology of the species. Terpenes and terpenoids, included in the essential oils of plants, are compounds that are necessary for the survival of plants in each particular biocoenoses and at the same time, they are necessary for the growth and development of plants [11]. Each ecotone of field mint is characterized by its own level of content of individual groups of components. These compounds have significant structural diversity with high intra-specific variability, which allows plants to protect their habitat with certain ecological characteristics, including natural enemies, competitors, and mutualists [12].

Up to 65 components were found in the composition of mint essential oil, all components with a content of more than 0.1% of the total amount were easily identified by the retention time and mass spectra (Table 2). Cyclic terpenes and terpenoids (65.2 and 59.8%, respectively) predominated in the composition of the field mint plants oil of mesohydrophytic and mesophytic biocoenoses, the soil moisture content of which is more consistent with the mesophilic ancestral ecology of the species,
while acyclic compounds accounted for 31.2 and 34.6 %. Simpler acyclic terpenes and terpenoids dominated in the field mint oil profile of xeromesophytic biocoenoses (55.3%). The shift in the activity of secondary metabolism in the direction of increasing the synthesis of simpler components as the species moves to new habitats that differ from those specific to ancestral forms is consistent with the position of the evolutionary advancement of species that synthesize simpler structures and less energy-intensive terpene compounds in competitive conditions, providing the population with a selective advantage [13, 14].

**Table 2.** Composition of essential oils of *Mentha arvensis* in the different ecological groups

| Compositions (%) | Ecological groups |
|------------------|-------------------|
|                  | Mesohygrophytes   | Mesophytes | Xeromesophytes |
| Acyclic monoterpenes |                   |            |                |
| β-Myrcene         | 2.26              | 2.23       | 4.26           |
| trans-Ocimene     | 0.09              | 10.95      | 22.3           |
| cis-Ocimene       | 0.10              | 11.14      | 23.00          |
| Linalool          | 0.18              | 0.17       | 0.43           |
| trans-Geraniol    | 4.11              | 0.41       | 0.15           |
| cis-3-Hexenyl isovalerate | 0.68 | 2.05 | 1.37 |
| cis-Geraniol      | 6.81              | 2.23       | 1.45           |
| Nerol acetate     | 9.51              | 2.58       | 0.70           |
| Geraniol acetate  | 2.02              | 1.53       | 1.56           |
| Total acyclic monoterpenes | 31.20 | 34.63 | 55.33 |
| Mono- and bicyclic monoterpenes |          |            |                |
| α-Pinene          | 0.27              | 0.88       | 2.20           |
| Sabinen           | 0.47              | 1.24       | 3.12           |
| β-Pinene          | 0.56              | 3.41       | 5.79           |
| 2-Carene          | 3.10              | 0.79       | 0.10           |
| α-Cimene          | 2.31              | 7.99       | 0.65           |
| Eucalyptol        | 2.63              | 7.38       | 18.43          |
| Limonene          | 0.38              | 1.73       | 1.45           |
| γ-Terpinene       | 52.31             | 24.55      | 1.87           |
| α-Terpinolen      | 1.40              | 8.88       | 2.10           |
| Isopinocamphone   | 0.17              | 0.36       | 0.30           |
| 1-Terpinen-4-ol   | 0.38              | 0.49       | 0.27           |
| α-Terpineol       | 0.20              | 1.33       | 2.49           |
| Total cyclic monoterpenes | 65.36 | 59.69 | 39.07 |
| Mono- and bicyclic sesquiterpenes |         |            |                |
| β-Caryophyllene   | 1.24              | 2.93       | 1.03           |
| Germacrene D      | 1.66              | 3.82       | 5.12           |
| Caryophyllene oxide | 0.32           | 0.51       | 0.28           |
| Total sesquiterpenes | 3.22             | 7.97       | 7.54           |
The study of the ratio of the quantitatively predominant components of the population terpene complex not only indicates the phenotypes but also shows how the variability of the components at the intra-population level is involved in the formation of pre-adaptive individuals with characteristics specific to individuals of the neighbouring habitat. It is known that the most likely function of plant terpenoids is the protection against biological enemies. This is either a direct effect on herbivores in the form of toxins and repellents, or indirect - through the attraction of predators and enemies of such herbivores [15, 16]. Thus, the essential oil of mint plants of all three ecological biocoenoses contained terpenes with a pronounced protective function - \( \gamma \)-terpinene, \( \alpha \)-terpinolen, trans and cis-ocimene, eucalyptol (1,8 cineol), which exhibit broad antimicrobial and insecticidal properties. The content of these components in the composition of plant oil increased in the following series:

Mesohydrophytic biocoenoses (55.1%) < mesophytic biocoenoses (62.9%) < xeromesophytic biocoenoses (71.6%).

At the same time, some volatile terpenes (for example, \( \beta \)-myrcene, cis-geraniol, nerol acetate), along with protective functions, perform an attractive function, since such compounds play an important role in the relationship between plants and insects. Secretory terpenes act as taste or olfactory attractants for a certain type of insect pollinators - obligatory plant- flowers visitors [17]. The concentration of these substances increases in the opposite direction:

Mesohydrophytic biocoenoses (18.6%) > mesophytic biocoenoses (7.0%) > xeromesophytic biocoenoses (6.4%).

It should be noted that the assignment of strictly defined functions to specific terpenes is not correct, since even within the same organ of the plant, many very similar terpenes are produced. Most often, terpenes with certain functions are found in the form of complex mixtures of many related compounds in rapidly evolving species, suggesting that it is the terpenes diversity that gives an advantage to this species.

4. Conclusion

Thus, the total content of essential oils in the plants of field mint \( M. \ arvensis \) L is quite informative at the inter-population level, since it allows us to identify its relationship with a certain ecological environment, the influence of which is determined by the moisture supply nature of the ancestral and modern ecology of the species. The output of essential oil from the aboveground part of mint plants was maximum during the mass flowering phase and amounted to 2.7±0.08% in mesohydrophytic biocoenoses, 2.3±0.09% in mesophytic and 1.3±0.11% in xeromesophytic biocoenoses. Cyclic terpenes and terpenoids (65.2 and 59.8%, respectively) predominated in the composition of the field mint plants oil of mesohydrophytic and mesophytic biocoenoses, the soil moisture content of which is more consistent with the mesophilic ancestral ecology of the species, while acyclic compounds accounted for 31.2 and 34.6 %. Simpler acyclic terpenes and terpenoids dominated in the field mint oil profile of xeromesophytic biocoenoses (55.3%). The essential oil of mint plants of all three ecological biocoenoses contained terpenes with a pronounced protective function - \( \gamma \)-terpinene, \( \alpha \)-terpinolen, trans and cis-ocimene, eucalyptol (1,8 cineol), which exhibit broad antimicrobial and insecticidal properties. The concentration of these compounds was maximal in plants of the xeromesophytic biocoenoses. While the maximum content of volatile terpenes (\( \beta \)-myrcene, cis-geraniol, nerol acetate), which perform an attractive function (ensures the relationship of plants with insects), is recorded in the mesohydrophytic biocoenoses. The adaptation of mint plants to different ecological conditions is ensured by the shift in the activity of secondary metabolism towards increasing the synthesis of simpler in structure and less energy-intensive components, which provides the population with a selective advantage as the species moves to new habitats that differ in ecological conditions from those specific to ancestral forms.

Acknowledgments

The reported study was carried out in accordance with the MBG RAS Research Project No 18-11802149011-5.
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