The Content of Biologically Active Substances in the Aboveground Phytomass of Catmint (*Nepeta Cataria* L.) in Ontogenesis

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***Abstract*—The need for a systematic phytochemical studying of *Nepeta cataria* L. is related to solving the problem of using of this valuable plant resource. The purpose of the work was to study the dynamics of the accumulation of biologically active substances in the aboveground phytomass of *N. cataria* L. in the Republic of North Ossetia-Alania. In years 2008-2013 the study of *N. cataria* L. was carried out in a culture on the basis of standard methods. The aboveground phytomass of *N. cataria* L. contains a variety of biologically active substances. A study of the dynamics of the content of the main biologically active substances in ontogenesis showed that during mass flowering, the maximum content is reached for essential oils, total acidity, ascorbic acid, and the content of tannins. The content of individual biologically active substances did not depend on the ontogenesis phase and remained at the same level during the growing season. There was established the relationship between the biologically active substances content and the weather and climate conditions of the year: direct – with average air temperature and inversely proportional – to the amount of precipitation.**

Keywords— Nepeta cataria* L., *phenological phases of development, biologically active substances, antibacterial activity

I. INTRODUCTION

The principles of a unified state system of environmental monitoring are laid in the study of modern vegetation cover in Russia. A significant place is given to assessing the state of phytobiota, studying the dynamics of seasonal and ontogenetic changes. This is due to the ratification by Russia of a number of international documents regulating the conservation and sustainable use of plant genetic resources for food and agriculture [1, 2].

Studies related to the expansion of the range of plant species for use in food have priority in pharmaceutical, cosmetic and other industries. The possibilities of introducing of biological resources into the culture and practical use of many species of the *Lamiaceae* L. family, including species of the genus *Nepeta*, are being actively studied. The increased interest is due to their chemical composition, mainly a high content of essential oils and other biologically active substances (BAS) [3-6].

Species of *N. cataria* L., grow on the territory of North Ossetia-Alania: *N. biebersteiniana* (Trautv.) Pojark.); *N. cyanea* Stev.; *N. komarovii* E. Busch.; *N. pannonica* L.; *N. somkhetica* Kapell.; *N. supina* Stev; *N. czegemensis* Pojark. – a regional endemic that occurs in the steppe ecological-phytocenotic group. The species *N. cataria* L. in North Ossetia-Alania is small and protected. However, this species is widely cultivated in Western Europe, the USA, the CIS, and also in some regions of Russia as a valuable essential oil and food plant [7–9].

The chemical composition of *N. cataria* L. is being actively studied. Biologically active substances are represented by tannins, flavonoids, terpenoids, including saponins. The main group of biologically active substances of *N. cataria* L. is an essential oil, which includes Nepalactone, 1.8-cineole, α-pinene, α-humulene and other terpenes [10-13].

In *Lamiaceae* family essential oils accumulate in essential oil glands. They are a mixture of fragrant volatile substances and belong to various classes of organic compounds (hydrocarbons, alcohols, aldehydes and ketones, phenols, lactones, acids, ethers and esters, etc.). Essential oils increase plant immunity, stimulate tissue repair processes, serve as a reserve of substances that are consumed during flowering, and also attract insects for pollination, are raw materials for the pharmaceutical industry [14].

In folk medicine, the herb *N. cataria* L. is used to treat chronic bronchitis, catarrh of the stomach, diseases of the hepatobiliary and female reproductive systems. In the experiment, the herb extract *N. cataria* L. has an antispasmodic, antidepressant, sedative effect; exhibits antimicrobial and immunostimulating activity, has high antioxidant properties [15, 16].

A systematic phytochemical study of *N. cataria* L. is necessary in order to solve the problem of using this valuable plant resource and determine the cumulative dynamics of the biologically active substances in ontogenesis.

To establish the cumulative dynamics of biologically active substances in the above-ground phytomass of catmint (*N. cataria* L.) in the North Ossetia-Alania.

II. Experimental

In years 2006-2012 there were conducted an expeditionary survey and resource studies on the territory of North Ossetia-Alania to identify fishing areas and potentially productive lands of economically valuable species of the Lamiaceae L. Resource studies were carried out by the route method according to A.I. Schröter (1986) [17]. Belonging to the ecological regime was evaluated according to D. Tsyganov. (1983) [18].

In years 2008-2013, the study of *N. cataria* L. was carried out in a culture on the basis of the collection nursery of the Botanical Garden Research Institute of Biotechnology FSBEI HE "Mountain State Agrarian University". The garden is located in a forest-meadow zone on soddy-gley soils with a slightly acidic soil reaction, pH = 4.9-5.2. Weather and climatic conditions are given according to the data of the state meteorological service of the North Ossetian Republican Center for Hydrometeorology and Environmental Monitoring.

The conditions of 2008 were marked by insignificant deviations of temperature from the norm, with the exception of March (+ 5.3 °C) and April (+ 2.1 °C). The amount of precipitation from March to August was significantly lower than the long-term average data, which also had an adverse effect on the growth and development of some plants.

The conditions of the growing season of 2009 were more stable and favorable (according to meteorological conditions) for plant growth; in May, July, August and September, 238 mm, 84.6 mm, 163.5 mm, 198.3 mm of precipitation fell respectively.

In 2010, the summer period was generally moderately hot. The annual precipitation was 910 mm, or 6.3% below the norm; August and September were arid. At the beginning of the growing season in the foothill zone, it rained heavily.

The 2011 year turned out to be warmer and wetter than usual. During the year, the air temperature was 16.17 °C, which is 2.8 °C higher than normal. The annual precipitation amounted to 1,008.4 mm, or 3.7% above the norm.

In 2012, the average air temperature for the year was 10 °C, which is 1.6 °C higher than normal. Annual precipitation was 10.8% below normal.

Weather and climatic conditions on average for 2008-2012 were favorable both for the formation of above-ground phytomass and for the accumulation of biologically active substances. The yield of green mass of catmint was determined according to D. Muravyov, et al., 2008 [19]. Sampling was carried out according to standard methods [20, 21]. Samples for research were stored in the laboratory in accordance with the requirements of GOST (State Standard) 17768-90 [22]. Samples were taken in phases: the beginning of regrowth, budding, flowering, and fruiting. There was hold 5 repetition of experience. Chemical analyzes were carried out in the laboratory of the Research Institute of Biotechnology of FSBEI HE “Gorsky GAU”. The following were determined: essential oils by distillation from plant materials with water vapor of the essential oil and subsequent measurement of its volume, expressed as a percentage in relation to absolutely dry raw materials, tannins by the method of potassium oxidation with permanganate in the presence of indigosulfonic acid [23]; total titratable acidity according to A.I. Ermakova (1987) [24]; flavonoids, anthracene derivatives (anthraglycosides) according to the methodology of the State Pharmacopoeia (1990) [20]; ascorbic acid according to GOST (State Standard) 7047-55 [25]; carotene according to GOST 13496.17-95 [26]; saponins according to A.V. Kiseleva et al. (1991) [27]. The content of biologically active substances was calculated on an absolutely dry mass of raw materials. The antibacterial activity of plants was determined by diffusion into agar according to M. Gulluce et al. (2007) [28].

Statistical processing of the results was carried out using the Microsoft Excel computer program by using student’s t-test.

III. Results and discussion

It has been established that *N. cataria* L. in the North Ossetia-Alania is found in the steppe, mid-mountain forest belt, along alpine meadows, in the valleys of streams and along riverbanks, among shrubs. It belongs to the synanthropic marginal-shrub meadow, ecological-phytocenotic group. *N. cataria* L. in relation to moisture is a mesophyte, in relation to nutrition – mesotroph, eutrophic, in relation to light – heliotroph (photophilous), semigeliofit (shade-tolerant).

N. cataria L. is a perennial herbaceous, highly branched and densely leafy plant up to 60 cm tall. The stems are ascending, branched, leafy, and pubescent. The cross section is concave-quadrangular.

The leaves are oblong-ovate or lanceolate on the petiole or almost sessile, scalloped on the edge. The flowers are bisexual, five-membered, two-lip, collected in thick complex semi-umbrellas, collected at the ends of the stem and branches in the form of a brush, the corolla is purple-white. Stamens glabrous, with violet-blue anthers lying above the upper lip. The type of inflorescence is frandulose-bracious, refers to a complex elongated polytelic thyrsus, consisting of 7-13 pairs of cymoid partial inflorescences, representing double dichasia.

Budding of *N. cataria* L. occurs in early June, and flowering in mid-June – August. Fruits in July-September. The fruit is a smooth quadrangle. The mass of 1000 seeds is 0.5-0.7 g.

The productivity of the aboveground phytomass of *N. cataria* L. in the studies varied from 0.1 to 0.4 kg/m^2.

The study of the BAS group in the aboveground phytomass of *N. cataria* L. began with an assessment of the dynamics of the content of essential oils (Table I).

The content of essential oils in the ontogenesis of *N. cataria* L. varies. This is due to the physiological role that they play in plant metabolism. In the first half of the growing season, when there are active processes of growth and development, their content increases.

TABLE I. THE DYNAMICS OF THE ACCUMULATION OF ESSENTIAL OIL IN THE AERIAL PART OF *N. CATARIA* L., DEPENDING ON THE PHASE OF VEGETATION

Years	Aboveground phytomass collection, kg/m^2	Start of vegetation	Budding-the beginning of flowering	Simultaneous flowering	Fruiting	Average
2008	0.31*	0.37*	0.39	0.44	0.17	0.34
2009	0.42*	0.39	0.40	0.50*	0.20	0.37
2010	0.34	0.38	0.43*	0.52*	0.24*	0.39
2011	0.22*	0.32*	0.35*	0.44	0.19	0.32
2012	0.33*	0.40*	0.43*	0.60*	0.15*	0.39
2013	0.29	0.35*	0.44*	0.29*	0.16	0.31
Average	0.27	0.37	0.40	0.46	0.19	0.36

* - The difference in years is significant at $P \leqslant 0.01$

At the beginning of the growing season, from the period from the beginning of regrowth to flowering, the content of essential oils in *N. cataria* L. is in the range from 0.37% to 0.40% respectively.

During the period of mass flowering, the content of essential oils becomes maximum and reaches an average of 0.46%. Depending on the climatic conditions of the year, this value varies. In our experiments, from 0.29% in 2013 to 0.52% in 2010.

During fruiting, the content of essential oils in tissues is reduced to a minimum and on average does not exceed 0.19%.

The species *N. cataria* L. is characterized by accumulation, in addition to essential oils and other biologically active substances. The dynamics of their content are presented in Table II.

The total titratable acidity reflects the amount of hydrogen ions (pH) in the vegetable juice, i.e. active acidity. In the tissues of *N. cataria* L. the total acidity varied from 1.93% during the budding-start of flowering phase in 2008 to 3.11% during the mass flowering phase in 2009.

An important component of biologically active substances in plants is carotenoids, which play the role of carriers of active oxygen. These pigments are concentrated in plant plastids in the form of water-soluble protein complexes or are in the form of oil droplets [19].

TABLE II. THE DYNAMICS OF THE ACCUMULATION OF BIOLOGICALLY ACTIVE SUBSTANCES IN THE GRASS OF *N. CATARIA* L. IN DIFFERENT PHASES OF DEVELOPMENT

Index	Plants vegetation phase		
	Budding-the beginning of flowering	Simultaneous flowering	Fruiting
Acidity, %	2.25±0.42	2.88±0.36	2.41±0.54
Carotene, mg%	1.86±0.07	1.90±0.15	1.83±0.26
Ascorbic acid , mg%	29.94±2.7	42.05±2.8	28.00±3.1
Flavonoids, %	1.22±0.8	1.21±0.5	1.10±0.05
Anthracene derivatives (in terms of istezine), %	0.13±0.04	0.13±0.05	0.13±0.03
Tannins,%	1.25±0.02	0.99±0.02	0.89±0.03
Saponins (in terms of glycyrrhizin acid), %	0.26±0.03	0.25±0.03	0.25±0.03

In the tissues of *N. cataria* L., the carotene content did not depend on the ontogenesis phase and remained on average at the level of 1.86 mg%. To a greater extent the pigment content depended on the year of research. In 2011, the carotene content in plant tissues was minimal and averaged 1.69 mg%. In 2012, the average carotene content was 1.97 mg%.

Ascorbic acid – a component of antioxidant activity, is involved in the adaptation of metabolism to environmental conditions. The level of its content in the tissues of *N. cataria* L. varied depending on the ontogenesis phase from 29.94 mg% during the phase of budding – the beginning of flowering to 42.05 mg% during simultaneous flowering and decreased to 28.00 mg% in the phase of fruit formation.

A certain correlation of the ascorbic acid content with an increase in the average air temperature in June (r = 0.66 and r = 0.93) is traced and is inversely proportional to the amount of precipitation (r = -0.75 and r = -0.72).

Flavonoids are involved in the enzymatic processes of oxidation and reduction together with ascorbic acid. Their high biological activity is due to the presence of active phenolic hydroxyl and carbonyl groups in the molecule [29].

The ontogenesis phase did not depend on flavonoids and averaged 1.18%. To a greater extent the content of biologically active substances depended on the year of research. The flavonoid content in *N. cataria* L. increases with increasing average air temperature during simultenious flowering (1.09-1.3%), which is confirmed by the values of the correlation coefficient (r = 0.62, 0.94). A decrease in the flavonoid content was noted with a decrease for precipitation (r = -0.79).

An accumulation of anthracene derivatives was detected in the grass of *N. cataria* L. This is a group of phenolic compounds based on the anthracene core of varying degrees of oxidation and condensation of monomeric forms. They have antibacterial, radioprotective, antitumor activity, laxative effect [30]. The accumulation of anthracene derivatives (in terms of istezine) was practically independent of the ontogenesis phase and averaged 0.13 %.

The content of tannins in the tissues of *N. cataria* L. on average ranged from 1.25% during budding to 0.89% during fruit formation. The maximum content of tannins – 1.63% was in the phase of budding – beginning of flowering in 2012. The minimum content of tannins was in the phase of fruit formation in 2011 – 0.75%.

Saponins are protective factors in the relationship of plants with other organisms, as well as raw materials for the pharmaceutical industry. Saponins are complex organic compounds with a high biological activity of a glycosidic nature. They belong to the group of substances whose aqueous solutions form a stable foam, similar to soap [31, 32].

On average, over 5 years of research, the level of saponins did not change significantly depending on the ontogenesis phase and was at the level of 0.25-0.26%. Analysis of data by years of research showed that in some years (2008, 2010, 2012), the content of saponins was maximum during all phases of ontogenesis and amounted to an average in budding phase of 0.31%, and in the full bloom phase 0.28%, in the phase of fruiting – 0.27%.

A wide range of biologically active substances that are contained in the aboveground phytomass of *N. cataria* L. suggests that there is antimicrobial activity in the tissues of this plant.

A microbiological study of the antibacterial activity of *N. cataria* L. revealed antagonistic activity against opportunistic and pathogenic microorganisms – *Escherichia coli* and *Staphylococcus aureus*. In experiments on the study of antibacterial activity which were carried out by the method of diffusion of biologically active substances in agar, the average size of growth inhibition zones of *E. coli* was 15 mm, and the growth inhibition zones of *S. aureus* was 20 mm.

IV. Conclusion

The species *N. cataria* L. in North Ossetia-Alania belongs to the synanthropically marginal-shrub meadow, ecological-phytocenotic group. In relation to moisture, it is a mesophyte, in relation to nutrition – a mesotroph, eutrophic, in relation to light – heliotroph (photophilous), semigeliofit (shadow-tolerant).

The aboveground phytomass of *N. cataria* L. contains a variety of biologically active substances. A study of the dynamics of the content of the main biologically active substances in ontogenesis showed that during the period of mass flowering the maximum content of essential oils (0.46%), total acidity (3.11%), ascorbic acid (42.05 mg%), tannins (1.63%).

The content of individual biologically active substances did not depend on the phase of ontogenesis. These included carotene (1.86 mg%), flavonoids (1.18%), anthracene derivatives (in terms of issthesin – 0.130-0.133%), saponins (0.25-0.26%). The relationship between the content of biologically active substances, weather and climatic conditions of the year is traced: direct with average air temperature and inversely proportional to rainfall.

The antibacterial activity of *N. cataria* L. in relation to opportunistic and pathogenic microorganisms *E. coli* and *S. aureus* has been established.

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