Accumulation of biologically active substances in peppermint varieties

Liudmila O. Sushkova*, and *Vladimir I.* Trukhachev

RSAU-Moscow Agricultural Academy named after K.A. Timiryazev, 127434, 49, Timiryazevskaya Street, Moscow, Russia

Abstract. Biologically active substances (BAS) are substances that play an important role in the life of organisms. They are involved in various processes, such as metabolism, regulation of growth and development, homeostasis maintenance. We can get natural biological substances from various plants. Such BAS include essential oils, which are essential in the relationship of plants with the environment. The accumulation of essential oils is influenced, among other things, by plant life expectancy. The article considers the dynamics of the accumulation of essential oils and individual components of a perennial peppermint plant by the years of crop cultivation.

1 Introduction

Biologically active substances (BAS) are compounds that affect biological processes and can affect the body functions. These substances can be found in various sources, including plants, animals, and microorganisms. They are often used because of their potential benefits in the treatment of various diseases and health conditions.

Bioactive compounds are another term used to describe biologically active substances. These compounds can be defined as nutritious and non-nutritious substances present in food that can have a physiological effect on the body. They can be found in both plant and animal sources, and their potential benefits for human health are still being studied.

Synthesis of biologically active compounds, including natural products and pharmaceuticals, is an important and interesting area of research, since the large structural diversity and complexity of biologically active compounds make them an important source of raw materials for drug development. To obtain a wide range of biologically active compounds derivatives from the medicine point of view, it is important to develop effective synthesis methods [1].

For example, recently there has been an increased interest in the synthesis of biologically active hydroxybenzoic acids and their derivatives, including those containing nitrogenous heterocyclic fragments, the most common among BAS [2]. Methods of synthesis of nitrogenous heterocyclic compounds are becoming more complicated every year, because the existing general approaches show their inefficiency. In this regard, an important task of the chemistry of biologically active substances and especially medicines

* Corresponding author: sushkova@rgau-msha.ru

is the improvement of old ones and the development of new effective, economically affordable methods for the synthesis of BAS.

Nevertheless, in addition to chemical synthesis methods, it is possible to use natural synthesis methods, for example, occurring directly in plants. Plants are a rich source of biologically active substances that can potentially be used to change the growth of crops or to protect crops from diseases and pests.

It is known that essential oils of one crop can be used in agriculture against pests, diseases, and weeds of another crop [3] whose essential oil can be used in the next crop – so the agricultural industry begins to support itself from the inside.

The plant is grown to obtain high-quality raw materials, plant components, biologically active substances from it, and the essential oils included in the composition can be used as by-products - and here they can also be put into production and used for the cultivation of other crops [4]. Thus, hydrolates – by-products of hydrodistillation of essential oils of various plants - can be used as plant protection products [5, 6].

Among essential oil plants, peppermint is one of the most widely used in medicine due to the high content of biologically active substances of vasodilating, soothing, and analgesic effect [7].

Mint leaves contain up to 3% of essential oil. It consists of more than 200 terpenoid-type compounds [8]. The main component is menthone and menthol isomers. The ratio of these components in the oil primarily depends on the type and variety of plants. Nevertheless, external factors can play a significant role in this ratio: climatic conditions, soil, fertilizers, etc. [9, 10].

Peppermint has various pharmacological properties: antimicrobial, anesthetic, anthelmintic, antifungal, and antioxidant effects. These properties are closely related to the action of the main active component, menthol, which is of great industrial and pharmacological importance [11].

The cultivation zone of the crop and the geographical location directly affect the ratio of certain components in mint essential oil [12]. Thus, it was shown that in the more southern regions the content of essential oil is higher than in the northern ones, but the menthol content, on the contrary, is lower [13]. According to some studies, the accumulation of alcohols in peppermint oil decreases in humid hot climates, while the percentage of ketones increases [14].

When using chemical plant protection products, it is necessary to consider the action mechanism of each preparation. Often, chemical plant protection products can negatively affect the direction of the biochemical process in cultivated plants, accumulating or, conversely, reducing important biologically active substances in the plant, which can affect the ultimate goal for which this essential oil crop was grown [15].

The pharmacological activity of essential oil plants is determined primarily by the chemical composition of the essential oil components, and then by biological signs. Terpenoids, which are components of essential oils and have a wide range of effects on the human body, are of great interest for phytotherapy. The increasing growth of the use of essential oil raw materials in medicine requires a certain amount and stability of the components of essential oils to expect the necessary pharmacological effect [16].

As a result of our research, data were obtained on more than 20 components of peppermint essential oil. In this article we describe the main of these components.

2 Materials and Methods

Peppermint of three varieties was chosen as the object of research: Krasnodarskaya 2, Yantarnaya, and Chernolistnaya. In general, all the varieties we have selected belong to the varieties produced for raw herbal pharmacy materials; this correlates with the relatively low

menthol content in their essential oil and gives reason to attribute them to low-menthol varieties.

The research was carried out at the experimental site of the Botanical Garden of the FSBSI VILAR. The micro-field small-scale experiment was laid in three-fold repetition.

The yield of air-dry raw materials, the content of essential oil and its quality were determined at the Department of Chemistry of the FSBEI HE RSAU-MAA named after K.A. Timiryazev. The plant material was dried and the essential oil contained in it was isolated by a modified Ginsberg hydrodistillation method.

The quantitative and qualitative composition of the peppermint essential oil components was determined by gas chromatography with mass spectrometric and flame ionization detection on a gas-liquid chromatograph "Clarus 600 GC/MS", manufactured by "Perkin Elmer Lift and Analytical Sciences". The obtained mass spectra of all compounds were processed by the search engine "NIST" for the library of mass spectra "NIST/EPA/NIH, ver. 2-2005". According to retention indices, in accordance with the previously obtained data of the developed connection library, the connections included in the test sample were finally established. Those components whose content was below the detection limit of the device – <0.01%, were designated by us as trace or 0.

3 Results and Discussion

To assess the potential yield of a perennial peppermint crop in the conditions of the Moscow region, we used an array of long-term data obtained by us on crop yield on average for all five years of the field experiment.

As can be seen from Figure 3.2, over five years of research, the yield of peppermint of the Yantarnaya variety was 157.2 g/m^2, and in other varieties this indicator was lower: 149.7 in Krasnodarskaya 2, and 152.0 g/m^2 in Chernolistnaya. This can be explained by the fact that the Yantarnaya variety was created in the Middle zone of Russia, while the Krasnodarskaya 2 and Chernolistnaya varieties are varieties of southern selection and in more northern growing conditions may have low productivity.

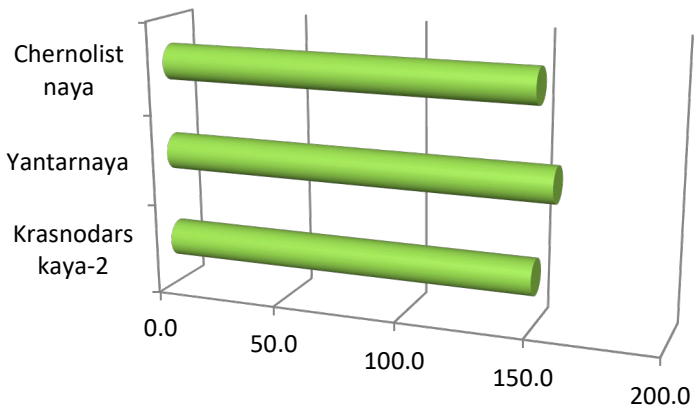

Fig. 1. The yield of peppermint plants (average for 5 years of cultivation), g/m^2.

The yield of the Krasnodarskaya 2 variety differs more strongly against the background of other studied varieties, since this variety has been greatly degenerating over the five years of vegetation in the Moscow region.

It can be concluded that for the cultivation of peppermint in the long term without the use of agrochemicals under conditions of the Non-Chernozem zone of the Moscow region, the Yantarnaya variety is generally more suitable for use as a perennial crop than the other varieties studied.

The quantitative and qualitative composition of the main components of peppermint essential oils is shown in Figures 2-5.

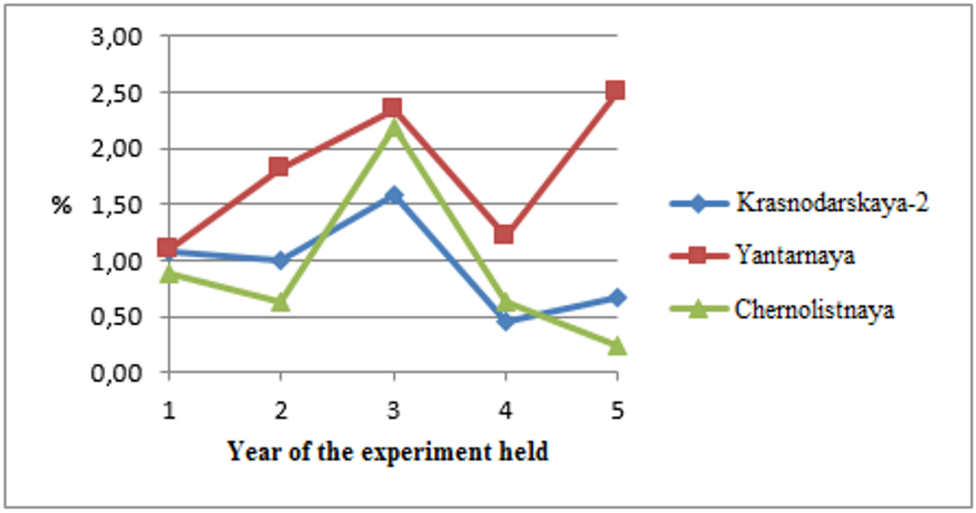

Fig. 2. Change in the limonene content in peppermint essential oil, %.

As is known, limonene is the compound from which the synthesis of terpenoids begins, leading to the menthol formation. Pulegone is synthesized from limonene, and expends on formation of menthofuran and menthone, where the latter one goes to the synthesis of menthol.

By the third year of cultivation of the Krasnodarskaya 2 variety, limonene reached its maximum value. This can be explained by the fact that it was by the third year that the peppermint of the Krasnodarskaya 2 variety reached the limit of its potential productivity, which is confirmed by the data on the yield of this variety that we previously considered.

The limonene percentage in mint of the Yantarnaya variety in the first year of cultivation of the crop was minimal, increasing by the third year of growth. In the fourth year, there was a sharp drop in the content of limonene, which is due to the weather conditions of this year, but by the fifth year there was a significant accumulation of this component and it reached its maximum for all 5 years.

The maximum percentage of limonene in the essential oil of peppermint of the Chernolistnaya variety was observed by us - as in the case of the Krasnodarskaya 2 variety - in the third year of cultivation of the crop, which, in our opinion, is explained by the achievement of the greatest bioproductivity of plants of these varieties in the third year of growth in the conditions of the Moscow region.

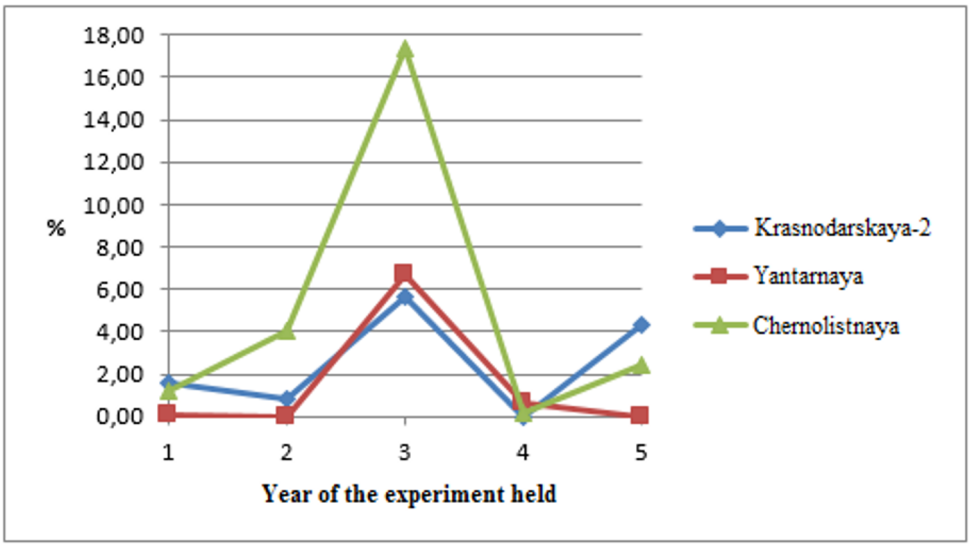

Fig. 3. Change in the content of pulegone in peppermint essential oil, %.

The content of pulegone, the precursor of menthone in the biosynthesis of essential oils, was also maximal in the third year of the crop growth and this can be traced in all the varieties we studied. The dependence of the contents of these two components on each other was observed. Thus, when the values of the pulegone content fall, it is found that the content of menthone on the contrary increases, and vice versa. The content of pulegone in plants of the Yantarnaya variety in individual years (2 and 5) was so low (below the minimum limit of the device detection limit) that it is equated to zero here. This means that our equipment registered an analytical signal for this component, but the value of the area of the corresponding peak was unreliably distinguishable from the noise of the device baseline. The maximum content of pulegone in the essential oil of peppermint of the Chernolistnaya variety was observed in the third year of the experiment and was the highest compared to other varieties.

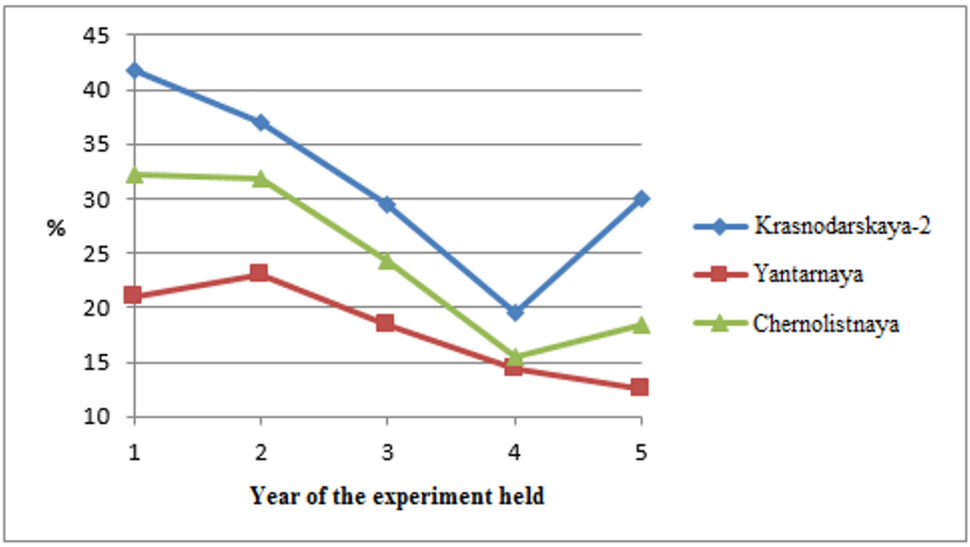

Fig. 4. Change in menthone content in peppermint essential oil, %.

With regard to such a component as menthone, it can be said that for the purposes of the chemical industry, its increased content is of interest as a raw material for the synthesis of menthol. From this point of view, the optimal content of menthone is the collection of essential oil in the first year of cultivation on the Krasnodarskaya 2 and Chernolistnaya varieties.

Nevertheless, for the purposes of the food and perfume industries, the increased content of menthone worsens the taste and quality of essential oil raw materials. Thus, for these purposes it is better to use the essential oil of plants of the fourth year.

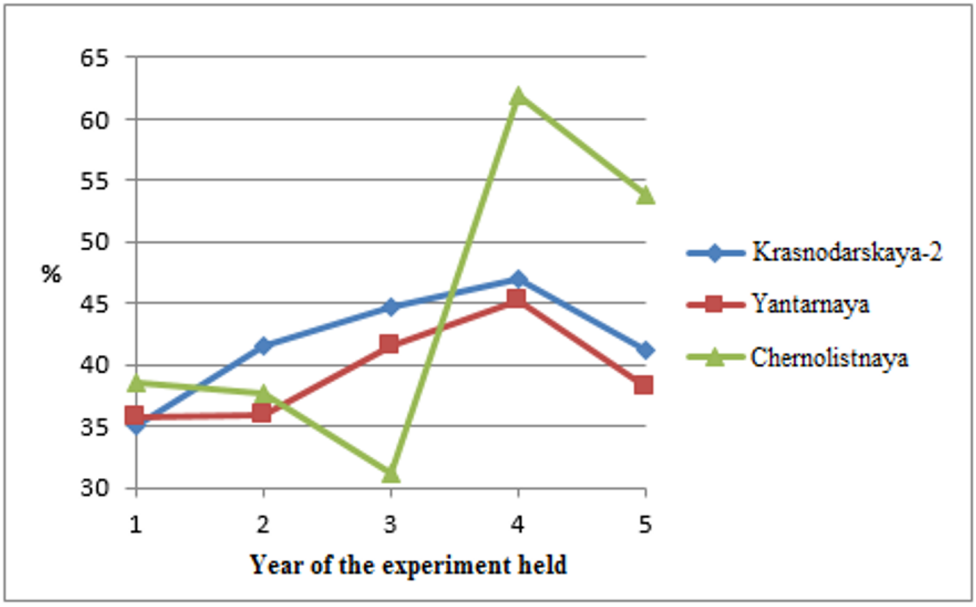

Fig. 5. Change in menthol content in peppermint essential oil, %.

The dynamics of the menthol accumulation - the most valuable component of peppermint essential oil - shows the opposite picture – with an increase in the period of cultivation of peppermint plants, the menthol content increases, reaching its maximum values in the fourth year of the experiment.

It is noted that in the years with the maximum value of menthone, the value of menthol content was the lowest for all the years of the crop cultivation.

4 Conclusion

Peppermint essential oil has a rich resource potential and a wide range of biologically active compounds of interest to various areas of the national economy. Therefore, each of them needs its own essential oil that is most balanced in all components and amounts of the main classes of substances. The value of each cultivated variety of peppermint is evaluated primarily by its yield and the quality of the raw materials obtained. Regardless of the fact that some of the varieties we selected were bred for universal use, in general, they all belong to the varieties produced for raw herbal pharmacy materials. Nevertheless, in our research, we have clearly shown that raw materials taken in certain years of crop cultivation can also serve as a source of biologically active substances such as menthol and menthone, valuable for the purposes of the medicinal, perfumery, and chemical industries.

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