Possibilities of Influencing the Biosynthesis and Accumulation of the Active Principles in *Chrysanthemum balsamita L S*pecies

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Abstract

The paper presents the way of influencing the processes of biosynthesis and accumulation processes of the active principles that are responsible for the therapeutical activity (volatile oil, phenyl propane derivatives) in the Chrysanthemum balsamita L. species, presented in Romania in two chemical infraspecific taxa.

There are presented the ways of intervening by growing technology and by specific treatments (nutritional area, plantation time, fertilization and use of growth regulators), that lead to obtaining of a medicinal vegetal product with a maximal content of active principles, thus being very important for the pharmaceutical and cosmetics industry.

Keywords: chrysanthemum balsamita, costmary

Introduction

The species *Chrysanthemum balsamita* L. (Asteraceae), with the common name costmary is known in Romania as two infraspecific chemical taxa, i.e. *Ch. balsamita* var. *balsamita* and *Ch. balsamita* var. *tanacetoides*, that are differentiated by the composition of volatile oil in herba: *Ch. balsamita* var. *balsamita* has cca. 85% camphor in the volatile oil, *Ch. balsamita* var. *tanacetoides* has cca. 60% L (-) carvone in the volatile oil. For this reason, the two taxa are known as *Ch. balsamita* chemovar. *camphora* and *Ch. balsamita* chemovar. *carvona*, respectively [1,2]. A third chemical taxon of *Ch. Balsamita* L, brought from the Botanical Gardens of Chisinew is known. It containins cca. 40% thujone in the volatile oil, which is in train of assimilation within the Research Laboratory for Medicinal and Aromatic Plants of Braşov [3].

Ch. balsamita L. is a very precious plant for therapy, due to its active principles, i.e. volatile oil and phenyl-propane derivatives. These active principles confer it both antimicrobial, stimulating and flavoring properties, and hepatoprotective and colagog-colleretic activity [2,4].

The paper presents the way certain technological factors influence the biosynthesis and accumulation of the active principles in the two chemical taxa of the *Ch. balsamita* L. species, in order to provide valuable data for breeding this species, that can be used in the pharmaceutical and cosmetics industry.

Material and Methods

The research performed within the Laboratory of Medicinal and Aromatic Plants of Braşov was focused on determining the way the amount of active principles varies in the two chemical taxa, *Ch. b. camphora* and *Ch. b. carvona*, depending on specific cultivation technology factors, i.e. the nutrition area, planting time, fertilization, use of growth regulators.

The propagation was made by the vegetative method, the species having large cloning possibilities. The propagation through the germination method was not satisfactory. The attempts of "in vitro" multiplication have given very good results, thus the research will continue in developing this way of propagation.

Studying the accumulation dynamics of the active principles in the two taxa, it was noted that their maximal concentration is reached at plant maturity, i.e. the blossoming period for *Ch. b. camphora* and the flower buttons formation period for *Ch. b. carvona* [5]. The plants cropping for the experiments have considered these aspects.

Although *Ch. balsamita* L. contains very numerous active principles (volatile oil, phenyl-propane derivatives, flavones, sesquiterpenic lactones, carotenoides, tannins, oligoelements) the paper presents only the influences that occur on the basic active principles. For the determinations, it has been used the steaming method (for volatile oil) and spectrometry (for phenyl-propane derivatives) [6].

Results and Discussions

Influence of the Plantation Time and Nutrition Area

For the two chemical taxa experiments were made with autumn (A) and spring (S) planting, using two variants for the nutrition area, i.e. 50 cm between the rows and 50 cm between the plants in a row (50/50) and 70 cm between the rows and 30 cm between the plants in a row (70/30).

The amount (%) of volatile oil and phenyl-propane derivatives, determined in green vegetal material (herba) was not significantly influenced by these technological conditions, but the yield of herba (kg/ha) is higher in the case of autumn (September) planting and for the 50/50 area, both in *Ch. b. camphora* and in *Ch. b. carvona.*

At an average 60% humidity of the vegetal material (herba), the average yield of volatile oil and phenyl-propane derivatives varies according to the planting time and distances (**Figures 1** and **2**).

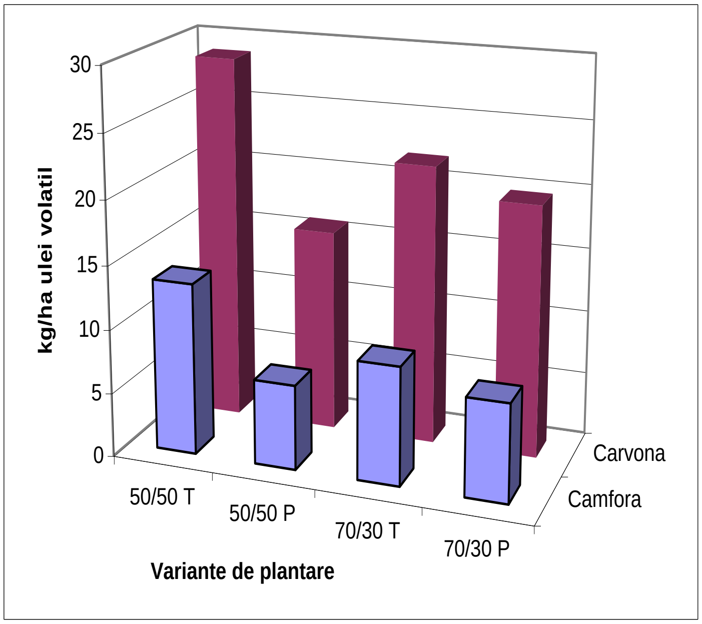

Figure 1. The influence of the plantation time and nutrition area - volatile oil

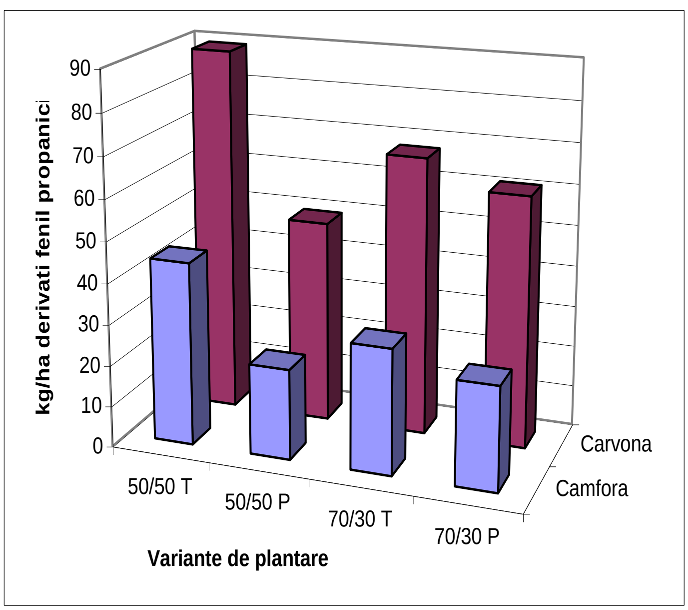

Figure 2. The influence of the plantation time and nutrition area - phenyl propane derivatives

It can be assessed that the maximal productivity in volatile oil and phenyl-propane derivatives is maximal for autumn planting, at the 50/50 cm distance, in both chemical taxa of *Ch. balsamita* L.

The Influence of the Fertilization

The soil, with its content in macro- and microelements, enhanced by the use of chemical and organic fertilizers, plays an essential role in plants growing and development, in the biosynthesis of the organic substances at cell level.

It is known the plastic role of nitrogen and the heavy damages that can occur in the plant metabolism in case of nitrogen absence or excess.

It is also known that phosphorus regulates the enzymatic and metabolic activity into the plant, in the same time conferring it diseases resistance.

The presence of potassium in the vegetal organism stimulates respiration and photosynthesis, cell permeability, phytohormones transport, and activates the activation of some enzymes having a specific role in the biosynthesis of the organic substances.

It has also to be mentioned the plastic and catalytic role of magnesium, that activates the metabolic and enzymatic processes [2].

The soil the experiments were performed had the following characteristics: nitrogen value 3.82; phosphorus value 85.7; potassium value 100.4; pH 6.1.

Considering the soil characteristics and recommendations concerning the optimal fertilizers doses for the medicinal plants on different soil types [7], we have used 6 different fertilizing options, i.e.:

V_1 - blank, without fertilizer;

V_2 - farm manure, 50 t/ha;

V_3 - phosphate, 90 kg P_2O_5/ha;

V_4 - nitrogen (NH_4NO_3), 50 kg N/ha;

V_5 - potassium, 60 kg K_2O/ha;

V_6 - complex fertilizer(NPK): 52 kg N/ha, phosphor 23 kg P_2O_5/ha, potassium 47 kg K_2O/ha (calculated according to [7]).

As it can be seen in the **Figures 3** and **4**, these treatments influence both the vegetative development and the plant content in active principles. It can be noted that when using manure, the vegetative mass is rich and the amount of the active principles is high. The presence of potassium and phosphor influence positively the biosynthesis of the active principles, even if the desired result is given when mixing them, thus enhancing reciprocally the stimulating action of the metabolic and enzymatic processes that take place at cell level, this also meaning the synthesis of the active principles. When using NPK fertilizer, it can be seen an increase in the amount of the active principles, compared to the blank (e.g. in *Ch. b. camphora* the volatile oil content is 0.91%, vs. 0.80% in the blank and the phenyl propane derivatives are 2.49%, vs. 2.35, respectively).

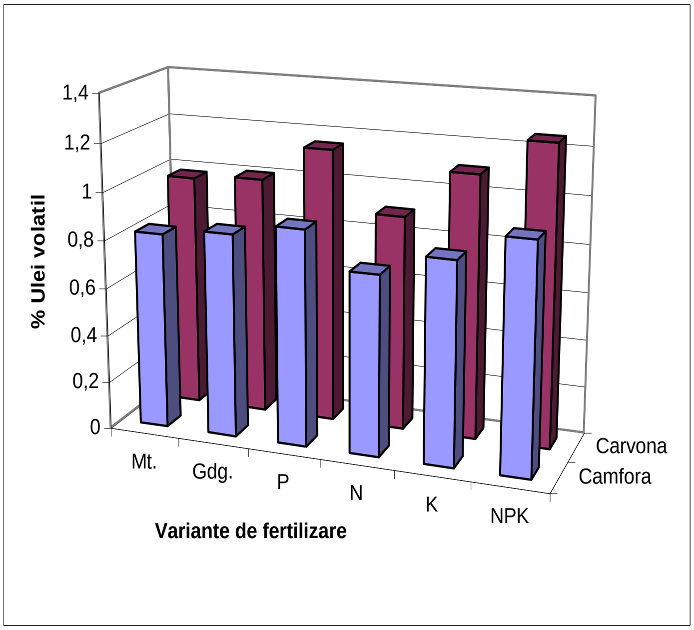

Figure 3. The Influence of the fertilization - volatile oil

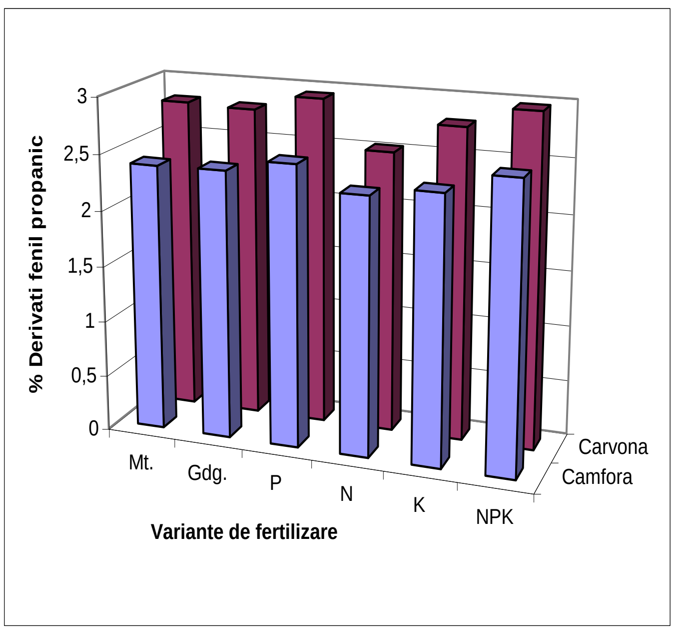

Figure 4. The Influence of the fertilization - phenyl propane derivatives

The analysis of the active principles was made on leaves and inflorescence, with 10 - 12% humidity.

The Influence of the Growth Regulators

Growth regulators are organic substances (other the nutrients), that, in small amounts stimulate, inhibit or modify the physiological processes in plants [8]. These substances, also named vegetal hormones or phytohormones, are self-synthesized by plants, participate separately or together to the metabolic processes and are active in different vegetation stages. Beside the natural way of preparation, they can also be chemically synthesized and then used for stimulating or inhibiting certain biochemical or evolution processes.

Growth stimulators influence plant development by: the stimulation of the germination and of the DNA and RNA synthesis; the production of lateral ramifications and additive buds formation, stimulating their growth on roots or stems; the rejuvenation of the aged tissue; the acceleration of blossoming. The best known stimulators (both natural and chemically synthesized) are the auxines, gibberellins and cytoquinines [8].

Growth inhibitors are substances that prevent the seed germination (proper or from other plants) and stop the development and the cell division, causing plant aging. They are known as antiauxines and antigiberrelins. The best inhibitor is the abscisic acid.

Retardants are more interesting from the point of view of research. They are differentiated from the inhibitors by not producing malformations. They have a moderate action of slowing plant growth, without blocking irreversibly the vital metabolic processes and without modifying plant viability.

The activity of some retardants is limited, because they can be modified in time by the plant into a product without inhibitory action.

Retardants, by the action they have until the cell metabolism usually stop the development of the vegetative organs (stems, offshoots), stimulating blossoming and fructification. Some retardants stimulate roots forming, stolons shortening, tubers forming, leaves thickening and greening, increasing the amount of chlorophyll and carotenoides, the accumulation of pigments, vitamins, mineral elements and biologically active substances.

By using the retardants (that stop the vegetative growth), the energetic substances can be sent and distributed towards the organs secreting biological active substances (leaves, flowers, roots).

Retardants increase plant resistance to diseases, pests and climatic factors. They also determine the accumulation of the substances that confer plants taste, scent and color, including the active principles.

The retardants used on a large scale are: Alar, Cicocel (CC), AMO 1618, Ethrel, Fosfon D, Alden and derivatives of the α-naphthylacetic acid (NAA).

In previous experiments on *Menta piperita* (1961), by using the methylic ester of the α-naphthylacetic acid (NAA), it has been obtained an increase of the volatile oil amount and its content in menthol [2].

Based on the existing information, were initiated varying the doses, application mode and time and considering the specific characters of *Ch. balsamita* L., three experiments of using growth regulators for the two specific taxa of *Ch. balsamita* L.[2]. These experiments were performed in vegetative pots, in three repetitions, having a blank for each of the two plant varieties.

The plants were treated with: gibberellin solution 100 mg/L, cicocel solution 0.5%, NAA solution 50 mg/L.

Following the treatments, there have been noted some differences in the vegetative stage of the plants.

The plant samples were cropped at maturity and the analyses were performed on dried samples - leaves and flowers - with 10 - 12% humidity.

The **Figures 5** and **6** present the way the phytoregulators influence the biosynthesis of the active principles of the two varieties of *Ch. balsamita* L.

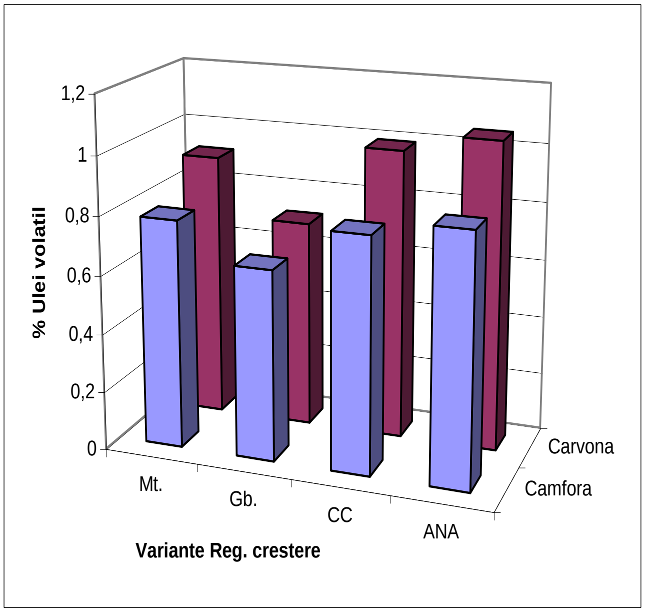

Figure 5. Influence of the growth regulators - volatile oil

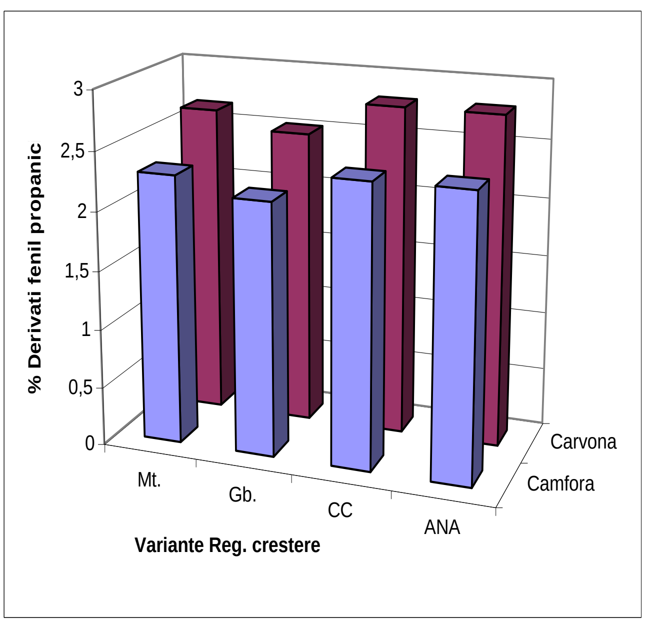

Figure 6. Influence of the growth regulators - phenyl propane derivatives

The treatment with gibberellins decrease both the amount of volatile oil and the content in phenyl propane derivatives, compared to the blank, while the treatment with Cicocel and NAA produce a sensitively higher amount of volatile oil and phenyl propane derivatives.

Conclusion

1. Biosynthesis and accumulation of the active principles in *Ch. balsamita* L. are directly correlated to the plant vegetative evolution, the biochemical transformations being influenced by several factors (climatic, agro-technical, ecological, etc.)

2. The experiments performed have demonstrated that the processes of biosynthesis and accumulation of the active principles, representative in *Ch. balsamita* L. (volatile oil and phenyl propane derivatives) can be influenced, by determining the optimal living conditions correlated to the plant necessities and by adding metabolism stimulating substances.

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