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COMPARATIVE PHYSIOLOGICAL AND BIOCHEMICAL RESEARCH OF TAXA OF THE *SALVIA* L. GENUS

- Abstract -

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INTRODUCTION

Of all new substances marketed during 1981-2006 about 30% are synthetic, the other 70% are natural substances or related to them. Approximately 80% of the world population uses herbal medicine recommended treatments and aromatherapy. Therefore we can say that it is necessary to convince doctors that traditional remedies and plants can be a source of new concepts for treating diseases and new drugs. Currently is considered to have been purified and characterized over 35,000 active substances in plants.(Burzo and Toma, 2012).

The *Salvia* genus includes about 900 species (medicinal, culinary, ornamental), spread across the globe, being recognized for its usage in traditional therapies used by civilizations such as the Chinese or the Romans.

The chemical composition of genus mainly includes oils, the predominant been the oxygenated monoterpenes and sesquiterpenele, but also polyphenols and flavonoids. Among the biological activities of these compounds we mention the anti-inflammatory, analgesic, antioxidant, antibacterial etc.

In Romania there are currently 15 species of the *Salvia* genus, including *S. officinalis* which is a medicinal plant included in the Romanian Pharmacopoeia, but also other species, spontaneous, that are used in traditional therapies.

GENERAL PART

The importance of the bioactive substances from medicinal and aromatic plants

The study of traditional remedies in recent decades has become one of the major objectives of OMS expects the integration of traditional medicine into public health programs. In this context and based on phytopharmacology data indicating aromatic herbs and essential oils as sources of therapeutic agents (Robu, 2011), a large number of studies have focused on the use of them.

As product quality is conditioned by the concentration of active principles, their accumulation depends primarily by the plant biochemical compounds, for which plant biochemistry studies are an indispensable element for modern pharmacognosy. In this situation, a new action was launched to return to the development of natural products of perfumery and cosmetics, pharmaceutical and food. In this way it will capitalize indigenous medicinal and aromatic flora, and the products will be used with more confidence in the therapeutic treatment either internal or, especially, external use.

This paper enrolls in this line of topics, searching, testing and proposing vegetable substances, given the odorous and therapeutic characteristics of wild plants and cultivated species. This approach aims to be a modest contribution to the knowledge of some taxa of the genus *Salvia* half spontaneous, spontaneous from the eastern area of the country, where they are widespread.

The species proposed for investigations, observations and research were carried out for three years, on their anatomy and micromorphology, physiological determinations, biochemical and microbiological testing.

Etymology, popular names, uses

The name of the plant comes from the Latin word *salvere = to save*, the name of *officinalis* referencing to the medicinal use of plants also. *Officina* was the name of a room from monasteries where they kept medicines and herbal medicines.

Croped by centuries and known as a source of food, but also for its medicinal properties, *Salvia officinalis* was described by Carl Linné in 1753.

Salvia officinalis contains essential oils in all its organs, being a well-known aromatic plant. Sage leaves have therapeutic use in human and veterinary medicine, and traditional culture. The volatile oil has antibacterial and antifungal properties, in large quantities it is used to preserve meat.

Spread of studied species of the genus *Salvia* in Romania

Most studied species are spontaneous species (less *S. officinalis*), spread almost all over the country. The exceptions are *S. aethiopis*, *S. nutans* and *S. ringens*, which have a limited spreading area, *S. ringens* is endemic to Romania, being found only in Dobrogea.

OWN RESULTS

Material and working methods

The purpose and objectives of the paper

The aim of the investigations was drawing phytochemical profile of some taxa of the *Salvia* genus (Lamiaceae), that are prevalent in eastern Romania.

Objectives

- observation of the histo-anatomical structure of the leaf and some aspects of micromorphology of leaf area, of some floral parts (sepals, petals), grain of pollen and mericarp of the taxa;
- Conducting physiological research regarding certain foliar indicators dynamic during ontogenetic cycle phenophases of plants;
- Determining the chemical composition of essential oils produced by investigated taxa;
- Investigating possible antimicrobial effects of essential oils extracted from plants at flowering time through microbiological testing “in vitro”.

Vegetal material investigated

The plant material investigated was represented by species belonging *Salvia* genus, of which 8 are spontaneous:*S. aethiopis* L., *S. austriaca* Jacq.,*S. glutinosa* L.,*S. nemorosa* L., *S. nutans* L.,*S. pratensis* L.,*S. ringens* Sibth.& Sm., *S.verticillata* L. and one crop: *S. officinalis* L.

The observations and investigations were conducted in three consecutive seasons, 2010-2012, in 3 ontogenetic moments: phenophase of vegetative growth, flowering phenophase and the fruiting plants.

Working methods

a) The methods used in the histo-anatomical research

The vegetal material (leaves) was cut and prepared using the histo-anatomical classical method. The observation and the photography of the samples were made by NOVEX microscope (Holland).

b) The methods used in the micromorphological research

The biological samples were prepared in advance by fixation, dehydration, drying, metallization and finally were analyzed with scanning electron microscope Tescan Vega SBH II., in vacuum, the acceleration of electrons of 27.88 kV.

c) The methods used in the biochemical research

- **The determination of the water content and dry matter** was done by gravimetric method (Boldor et al., 1982).
- **The dosage of the assimilating pigments** was done after Mayer–Bertenrath method (with changes made by Știrban and Fărcuș) (Boldor et al., 1982).
- **The determination of the polyphenolic compound** (flavonoid and polyphenol acids) was performed by high performance liquid chromatography (HPLC).

Analysis of the chemical composition of the volatile oils

- *Extraction of volatile oil* from plant material was performed by using a hydrodistillation device of Clevenger type (Burzo si Toma, 2012);
- *Analysis of volatile oils* was performed by GC MS method using an Agilant type device.

d) The methods used in the physiological research

To register processes of foliar photosynthesis, respiration and transpiration we used a LCI portable device for *in vivo* determination, while recording atmospheric moisture and atmospheric temperature with a portable thermo hygrometer, TESTO 625.

e) The methods used in the microbiological research

We used two methods: diffusion and microdilution method. The microorganisms used for testing were two pathogenic bacterial strains: *Escherichia coli* ATCC 25922 – Gram negative bacteria – and *Staphylococcus aureus* ATCC 25923 – Gram positive bacteria.

HISTOANATOMICAL OBSERVATION REGARDING THE UNIT LEAF OF THE TAXA OF THE *SALVIA* L. GENUS

The analysis of preparations made from leaves of 9 studied taxa of the *Salvia* genus, taxa emphasize common aspects and some common anatomical features for each taxon.

In general, the species investigated are characterized by the presence of many tector and secretory hairs, the secretory fits in the peltate hairs category (unicellular and / or multicellular) and capitate hairs (of various types). The

leaf is bifacial, and stomata are above the epidermis. In most species, the leaf is amphistomatic, at *S. aethiopis* can be hypostomatic.

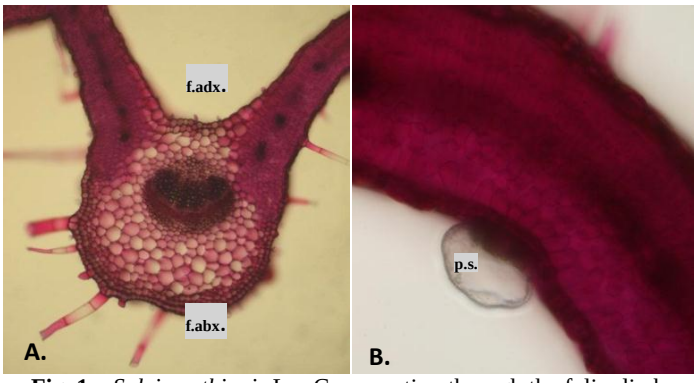

Fig. 1 – *Salvia aethiopis* L. - Cross section through the foliar limb
A – median rib, B – region between ribs (original photo);f. abx.= abaxially face; f.adx.= adaxially face; p.s. = secretory hair

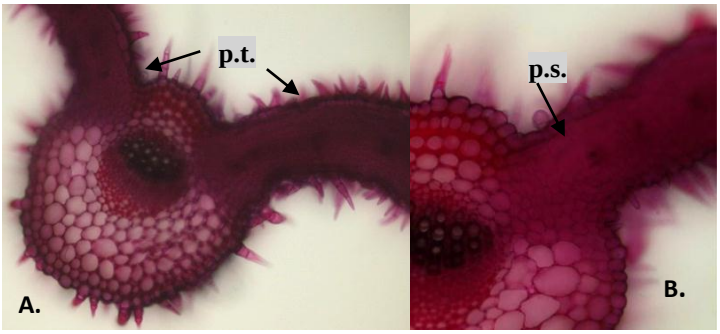

Fig. 6 – *Salvia nemorosa* L. - Leaf limb cross sections through the mid rib region (note numerous short multicellular tector hairs and rare secretory hairs) (original photo); p.t. = tector hairs; p.s. = secretory hairs

MICROMORFOLOGICAL OBSERVATION REGARDING THE SURFACE OF SOME VEGETATIVE ORGANS OR GENERATIVE ORGANS

Secretory hairs on leaf surface

There are two types of secretory hairs: peltate hairs and capitate hairs. Tector hairs are many and varied as morphology.

No structural differences were observed regarding secreting hairs on different organs analyzed; differences were only notified about the abundance per unit area, the highest density being in most taxa analyzed, the sepals and less, but present on petals.

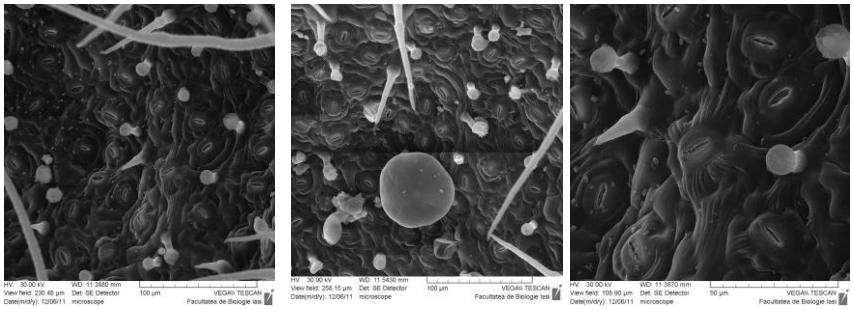

Fig. 7 - SEM microphotography of the leaf surface of *S. ringens* species: aspects of the upper epidermis (on both epidermis can be observed long tector hairs, multicellular hairs and secretory capitate hairs and peltate hairs) (original photo)

Micromorphological aspects of the calyx and corolla

The same morphological types of hairs found on the leaves were observed in this case also: secretory hairs – peltate and capitate hairs, and a very large number of tector hairs.

The abundance of peltate hairs on the sepals during the flowering phenophase and before the flowering is mentioned also for other *Lamiacee* (Corsi and Botega, 1999). When the sepals cover the tetrad of the nutles during their formation, the SEM images show destroyed hairs, that spilled out the contents of volatile oil.

Tector hairs are forked and varied as composition and appearance.

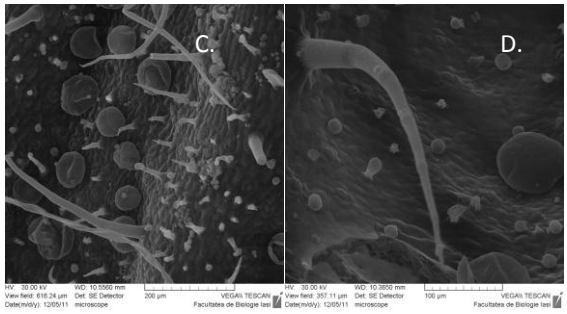

Fig. 12 - SEM microphotography of the sepals surface of *S. ringens* (original photo): C - aspect of the lower epidermis; D - aspect of the upper epidermis

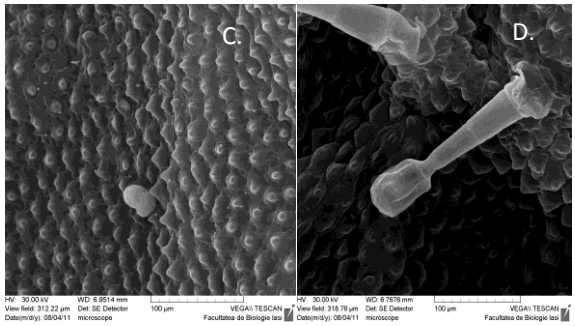

Fig. 18 - SEM microphotography of the petals surface of *S. ringens* (original photo)

Determination of the degree of hydration and the concentration of the assimilating pigments in the foliar tissues during ontogenetic cycle

The determination of the water content and dry matter in the foliar tissues

Although eco-physiological processes are genetically controlled, there is no exception to the law of variability (Murariu and Melinte, 2005), influenced by environmental factors (light, water, temperature). Therefore, the climatic conditions of the years 2010-2012, during which measurements were made, investigated taxa reveal in the dynamic, different levels of foliar tissue hydration.

The determination of the assimilating pigments content in the leaves

Chlorophylls undergoes a continuous process of biosynthesis and biodegradation, which is constantly renewing at a rate of 40% in 2-3 days (Burzo et al., 1999).

Among the analyzed taxa, in *S. nutans* the amount of chlorophyll a remained at high levels, above unit in every moment of analysis, the following species being *S. pratensis*. The minimum of chlorophyll a content was recorded in *S. aethiopis*, throughout the growing season, a similar situation can be mentioned at the taxon *S. austria.*

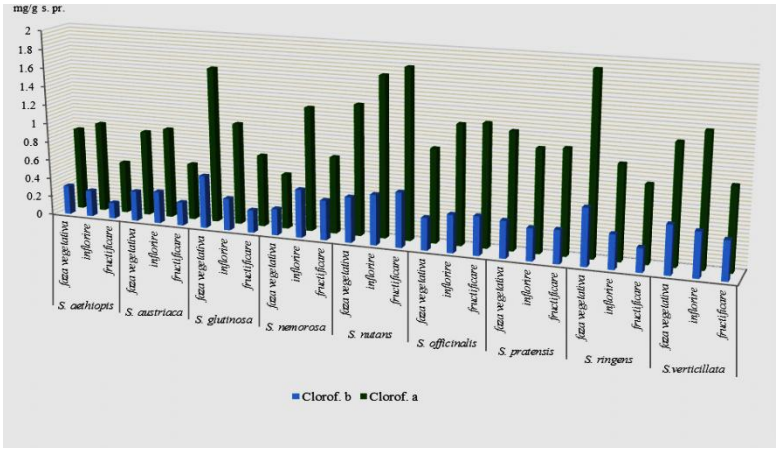

Fig. 3 Variation in foliar content of chlorophyll pigments in the investigated taxa of the *Salvia* genus, during the ontogenetic cycle

Following the dynamics of the second chlorophyll fraction, chlorophyll b, during phenophases of the ontogenetic cycle in the listed taxa, we can see, first, that for most of the investigated taxa the fraction of chlorophyll b has recorded the highest individual values in early ontogenetic cycle, a situation reported by other authors for taxa from Lamiaceae family (Andro et al., 2011).

In turn, carotenoid pigments also recorded for the analyzed taxa, specific quantitative variations induced by their biological nature and the amplitude of the light factor that was provided for the test plants.

Determining the intensity of some fundamental physiological processes at leaves level

Contributions to the knowledge of carring photosynthesis process in the foliar

By analyzing own data regarding the dynamics of photosynthesis during ontogenetic cycle stages for taxa taken into consideration, we find a general trend, an increase in the intensity of this process from the stage of vegetative growth to flowering period, after which there is a progressive reduction of that functional parameter. Our results are thus correlated with the data presented in the literature that emphasizes a direct functional link between the intensity of the photosynthetic process and photo assimilating foliar pigment content on the one hand, and the intensity of light radiation incident on the other hand (Trifu and Bărbat, 1997).

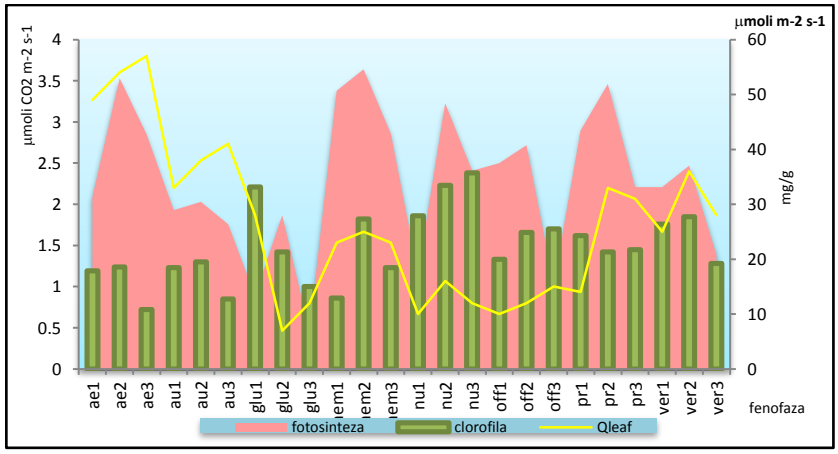

Fig. 8 Variation in intensity of photosynthesis process to taxa of the genus Salvia, during the ontogenetic cycle.* ae= *S. aethiopis*; au= *S. austriaca*; glu= *S.glutinosa*; nem= *S. nemorosa*; nu= *S. nutans*; off= *S. officinalis*; pr= *S. pratensis*; ver= *S.verticillata*; 1= vegetative phenophase; 2= flowering phenophase; 3= the fruiting phenophase; Q_{leaf}= lighting on leaf surface

It is important to know when the plant has its optimal ontogenetic photosynthetic, from a practical standpoint, at this time, the harvesting of the plant material being recommended at the maximum biosynthetic capacity to use for the extraction of active principles, in this case of essential oils.

Correlating the data obtained this way with those relating to the quantity and quality of essential oil produced by analyzed taxa, we observe that at the time of flowering, reviewed taxa that made intense photosynthetic process had biosynthesised a higher quantity of volatile oil, and especially of a higher quality.

Research on polyphenol fraction at the time of flowering

Phytochemical analysis by HPLC

In methanolic extracts have been dosed rosmarinic acid, chlorogenic acid, caffeic acid, p-coumaric acid, luteolin, luteolin-7-O-glucoside, apigenin, apigenin-7-O-glucoside. The analysis previously discussed was followed by spectrophotometric analysis of the flavonoid compounds and carboxylic polyphenol acid. According to the results, we can see that there is an accentuated variability in species dependent on quantity and not quality of the dosed compounds.

The dominant compound is **rosmarinic acid**, which is one of the most widespread acids caffeic derivatives (Lu and Yeap-Foo, 2002). Lamiaceae family, which includes *Salvia* genus, is characterized by the presence of rosmarinic acid, also known as „labiatenic acid" (Petersen and Simmonds, 2003; Zhou et al., 2011).

Gas chromatographic analysis of volatile oils

Volatile oils, called ethereal oils or essential oils, form a group of substances specific to secondary plant metabolism. The volatile oil samples analyzed were identified in a total of 96 compounds (common, frequent, present in a limited number of measurements respectively only one of these characteristics). Our observations on the dynamics of biochemical and physiological processes to taxa of the *Salvia* genus, submitted in a previous chapter, support the understanding of the essential oil production by these species.

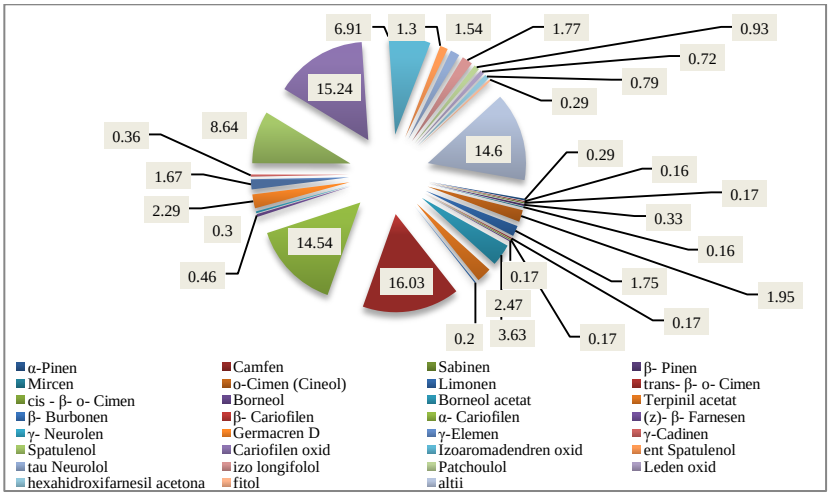

Fig. 16 The percentage of chemical composition of the essential oil extracted from whole plants *S. verticillata* during flowering phenophase

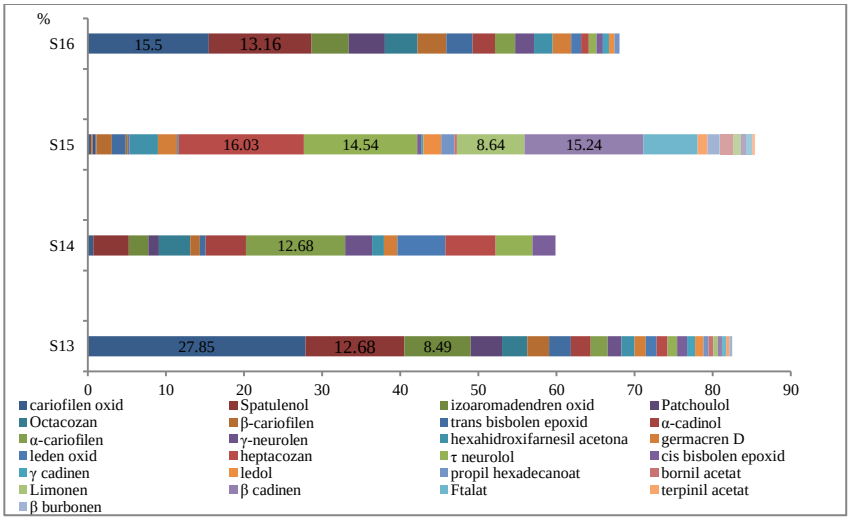

Fig 19 Intraspecific variation in the chemical composition of the essential oil produced by whole plants (S.verticillata) by phenophase ontogenetic cycle (S13= vegetative, S14= budding, S15= flowering, S16= fructification)

TESTING ANTIBACTERIAL EFFECT OF THE ESSENTIAL OILS PRODUCED BY INVESTIGATED TAXA

The plant products containing essential oils develop antimicrobial effects (bacteriostatic or bactericidal), basically they are used in therapeutics, showing also good properties as preservatives in the food industry.

Due to its lipophilic charactestic, the volatile oils easily penetrate the cell membrane, which may interfere thus indirectly in the metabolism of bacteria or fungi. Usually, their bactericidal activity is expressed as phenol coefficient.

- **Antibacterial testing by disc diffusion method**

In the case of the diffusion method, antimicrobial effect is determined by the appearance of clear zones around the outlined wells. In these areas antimicrobial substance inhibits the growth of bacteria. In the rest of the plate is observed bacterial growth area, where the active substance has not acted.

Essential oils collected from genus Salvia taxa analyzed were tested in three concentrations of 5, 10 şi 20 %.

- **Determination of minimum inhibitory concentration trough microdilution method**

For bacterial strain *Escherichia coli* test results showed that, for all six types of oils, the minimum concentration at which they showed antibacterial effect was about 0.5 % (Tab. 4). For bacterial strain *Staphylococcus aureus* results were different from one oil to another.

CONCLUSIONS

- The PhD thesis aims to highlight and discuss morpho-anatomical, physiological and biochemical characteristics, with taxonomic value of 9 taxa of the *Salvia* genus (8 spontaneous and 1 grown) from Romanian flora, taking into consideration also the possible antimicrobial effects induced by essential oils produced by studied taxa, bearing in mind the idea of future practical applications in the field of aromatherapy and more.
- The results provided by the morpho-anatomical research conducted confirms, detailed and complemented the literature, thus expanding the knowledge of foliageof the *Salvia* genus which vegetates in the eastern and south-eastern Romania;
- the leaf has a bifacial isofacial structure, in some cases with stomatal arrangement above the level of epidermis;
- presence of many unicellular or multicellular tector hairs on the surface of the foliar limb and ribs;
- on both sides of the leaf there are peltate and capitate hairs with uni-, bi- and multicellular secretory gland;
- Micromorphological data concerning the surface of pollen grains (exin ornamentation) and of the nutlet, emphasizes the idea that specific microscopic pattern is an important taxonomic index for representatives *Salvia* genus, one of the richest genera of the *Lamiaceae* family in the species spread across different areas of the globe.
- Biochemical and physiological measurements highlights the following:
- The degree of hydration and leaf dry matter content suggests, through the quantitative dynamics carried out, that analyzed species carry out fundamental physiological processes (photosynthesis, respiration, transpiration) with increasing intensity towards flowering time / fructification;
- The content of foliar assimilating pigments varies depending on the particular biological characteristics of the taxa investigated, on the specific metabolic level in the ontogenetic development of life cycle, as well as on changes in microclimatic factors (light, intensity and its quality, and ambient temperature), factors with significant variations in terms of carrying out a full annual life cycle
- The fundamental functional processes register specific dynamics, in obvious dependence on the variation of environmental factors where plant individuals live.
- Analysis of polyphenolic fractions from alcoholic extracts prepared from analyzed taxa show that the species *Salvia officinalis* can be considered, through practical results obtained, as the most valuable in terms of content of biologically active antioxidant principles, followed by *Salvia verticilata* and *Salvia glutinosa*; our results confirm the ideas stated by the literature that these two species have a real potential for medical use.
- Investigations on the composition of volatile oils obtained from the investigated taxa show a chemical composition of volatile oils that differ

quantitatively and qualitatively from one taxon to another, and from one to another phenophase;

- the number of components in the volatile oils is higher when they are extracted during flowering, in connection with their biological role in plant life, attracting pollinators;
- monoterpenes and sesquiterpenes give the general profile of these oils, printing the aromatic value specific for these plants, from *Lamiaceae* family;

➢ Testing the antimicrobial effect of essential oils produced by investigated taxa from the *Salvia* genus, allow us to conclude that the behavior of volatile oil samples analyzed confirmed the literature data, that shows their inhibitory effect evident from Gram positive bacteria compared to Gram negative;

AKNOWLEDGEMENT

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