VARIATION OF THE CONTENT OF α- AND β-THUJONE IN *SALVIA AETHEROLEUM* DUE TO DIFFERENT CULTIVATION TECHNOLOGIES

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Abstract: *Common or garden sage leaves (Folium salviae) contain volatile oil (at least 1.00%) and tanning materials (up to 8.00%). The main component of Common sage oil is thujone (30.00-50.00%). The leaves are sued in mouth conditions, in pharyngitis, amygdalitis, and laryngitis. It is widespread in gardens, as ornamental, meliferous, and medicinal plant. In Romania, they cultivate the local cultivar De Răsmireşti (BÂLTEANU, 1993). Aetheroleum Salviae is obtained by hydrolising Common sage leaves; it is a yellow or yellow-grenish, with neuter reaction, with strong camphor smell reminding that of Tanacetum (CIULEI, GRIGORESCU & STĂNESCU, 1993). Thujone has limited uses. This chemical compound is a neurotoxic substance that induces absinthism. This syndrome is characterised by addiction, hyperexcitability, and hallucinations. Because of this, FAO/WHO introduced, for the first time, in 1979, restrictions of the use of α- and β-thujone, limiting its sue as food or alcohol additives. Common sage volatile oil contains between 22.00 and 62.00% thujone (more (more in Italian or Bulgarian oil) depending on the group of compounds, alcohols expressed as borneol 2-165, terpenic hydrocarbons 35.00%, ketone 50.00%, and esters 3.00%. At present, scientists make new trials to see if Common sage (Salvia officinalis L.) is beneficial for patients suffering from serious cognition diseases such as Alzheimer's disease. A study published in February 2003 presented conclusions concerning the efficacy of Common sage in the treatment of light forms of Alzheimer's disease (AKHONDZADEH, NOROOZIAN, MOHAMADI, OHADINIA, JAMSHIDI & KHANI, 2003). The exact nature of Common sage effects was still obscure, but a previous trial carried out in laboratory conditions had revealed that Common sage can protect a substance destroyed in the Alzheimer's disease (PERRY, HOUGHTON, SAMPSON, THEOBALD, HART, LIS-BALCHIN, HOULT, EVANS, JENNER, MILLIGAN & PERRY, 2001). Common sage (Salvia officinalis L.) is a plant that, due to its biochemical constituents in Salviae Folium, Salviae Herba, or Salviae Aetheroleum, is a source of antioxidants and nutrients: calories, proteins, sugars, lipids, and alimentary fibber, vitamin K and iron (SANTÉ CANADA, 1995) with impact on blood lipids and sugars. The extract from green or dry leaves is of inner and outer use in both human (CONSTANTINESCU, 1976) and veterinary medicine (SINGH, SHARMA & JAIN, 1974; VERZAR-PETRI & THEN, 1975). Common sage is used in cooking, in the cosmetics industry and in the beverage industry (MYRSINI LAMBRAKI, 2003).*

Key words*: Common sage essential oil, α- thujone, β-thujone, row direction, oil quality*

INTRODUCTION

The variation of volatile oil content as well as of its components is pretty high in Common sage leaves and it depends on the harvesting period and on geographical location. According to CUCU et al. (1982), the leaves can contain, under certain conditions, up to 2.50% oil, this concentration being recorded between the bud and full bloom period.

After flower wilting, the amount of volatile oil gets below 1.00%. According to other authors, maximum concentration is reached during fructification (KOLODZEIYSKI, 1963).

If the main component, thujone, can get up to 50.00% in the Italian volatile sage oil, it is completely absent in the Spanish volatile sage oil (BRIEKORN & FUSCH, 1962).

According to CIULEI, GRIGORESCU & STĂNESCU (1993), the components of the Common sage essential oil are, gaschromatographically: α -thujone 34.20% and β-thujone

35.60%. α -thujone is the main constituent of volatile oils from different Common sage cultivars: *Artemisia absinthum*, *Artemisia vulgaris*, *Salvia officinalis*, *Salvia sclarea*, *Tanacetum vulgare*, and *Thuja occidentalis*.

GOAL OF RESEARCH

In this paper, we discuss the variation of the content of α- and β-thujone in the essential Common sage oil depending on cultivation technology in the soil and climate conditions of the Didactic Station in Timişoara.

MATERIAL AND METHODS

The biological material we have used is the cultivar *De Răsmireşti* of the Common sage (*Salvia officinalis* L.) from the S.C. PRONATURA S.R.L. in Zalău (Bihor County). The seed comes from typical, vigorous plants with a high content of oil, with 90.00% purity, 65.00% germination, and the volume of 1,000 grains is 7.5 g.

Research was carried out within the Didactic Station of the Banat University of Agricultural Science and Veterinary Medicine in Timişoara (Timiş County, Romania).

The crop of Common sage (*Salvia officinalis* L.) was treated with chemical fertilisers with macroelements (nitrogen, phosphorus, potassium, etc.) in the autumn and in the spring upon soil tilling and during vegetation by extraroot fertilisation using foliar fertilisers.

The biological material was harvested from the two agri-funds of the experimental field, i.e.: A_1 – east-west plant row direction and A_2 – north-south plant row direction.

Common sage was harvested in April, in the bud stage.

Extraction of volatile Common sage oil and isolation of compounds of interest – α-thujone and β- thujone was done from *Salviae herba* and dried *Salviae folia*, respectively. Harvesting and conditioning of Common sage was done according to the standards in application STAS.

Active substance content and dosage of essential Common sage oil were monitored in the laboratories of the S.C. Laboratoarele Fares Bio Vital S.R.L. in Orăştie (Hunedoara County) (ISO 9001:2000).

The results of the present study are part of a doctoral programme titled *„Ways of improving cultivation technologies of Common sage to increase herba, folia, and volatile oil content of Salvia oficinalis L."* and financed by the Ministry of Education, Research, Youth and Sports through the I.O.D. U.S.A.M.V.B. Timişoara under the guidance of the distinguished Professor Valeriu Tabără.

RESULTS AND DISCUSSIONS

The crude vegetal material subjected to extraction can be fresh or dried, according to the requirements of the Romanian Pharmacopoeia 2008. Dosage of the essential oil was done through the vapour distilling method in which the vegetal product was pulverised and the corresponding amount of water stipulated in the standard STAS working methodology were introduced into the distillation balloon. After the distillation was over – the duration met the methodological requirements – we read oil content in millimetres and we reported it to 100 g of vegetal product.

Common sage is very demanding in light. Common sage plants grow well in semi-darkness conditions, but their volatile oil content is a little higher (COCIU, E., RACZ, G., 1962).

According to laboratory data, we could see that, from the point of view of the content of α- and β-thujone f Common sage oil from herba and folia of Common sage (*Salvia officinalis* L.), there are differences depending on the system of orienting plant rows (E-W or N-S).

The toxicity of the essential Common sage oil is debatable: on December 2, 2002, they published the "Opinion of the Scientific Committee on Food on Thujone", according to the Annexe II of the Directive 88/388/EEC (EEC, 1998): in the U.E., it is allowed to use thujone as food additive, but not in the U.S.A.; the ISO, 1997 Standard allows the use of 18.00-43.00% α- thujone and of 3.00-8.50% β-thujone.

Depending on the orientation of the Common sage rows – E-W or N-S – there are variations of the content of α-thujone and β-thujone in *Salviae herba* (Figure 1).

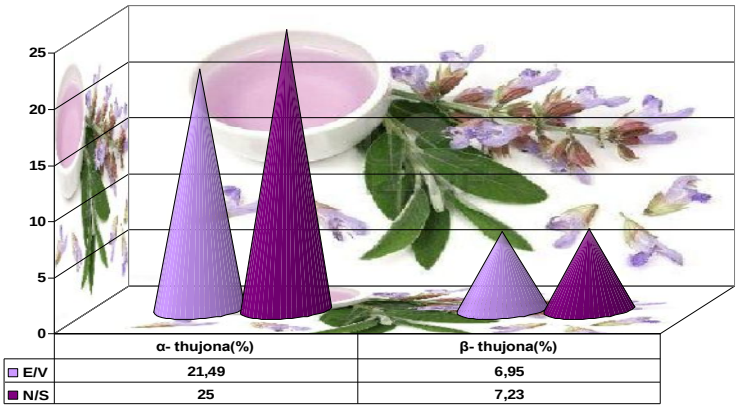


Figure 1. Variation of the content of oxygenated monoterpenes α- and β-thujone in *Salviae herba* depending on plant row orientation (E-W or N-S)

The lowest content of α-thujone, 21.49%, from the essential Common sage oil obtained from *Salviae herba*, under the conditions of Timişoara (2009), was in the Common sage plants cultivated over the direction E-W, followed by 25.00% of α-thujone, for the essential Common sage oil from Common sage plants cultivated over the direction N-S (Figure 2).

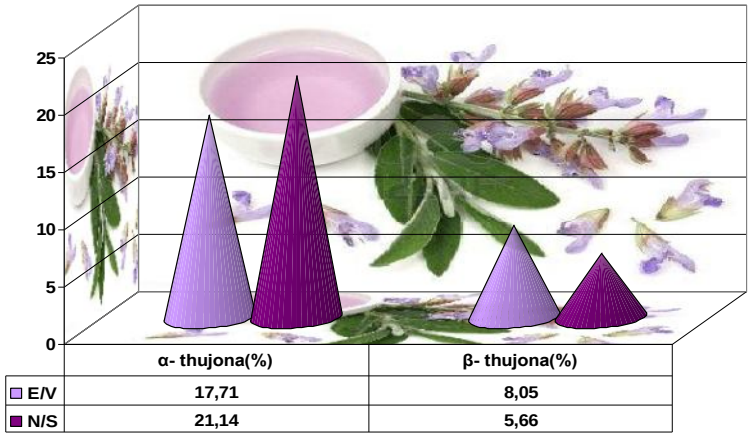


Figure 2. Variation of the content of oxygenated monoterpenes α- and β-thujone in *Salviae folia* depending on plant row orientation (E-W or N-S)

According to the Standard ISO/1997, content of α- thujone ranges between 18.00 and 43.00%, and content of β-thujone, the standard allows values between 3.00 and 8.50%.

The lowest content of α-thujone, 17.71%, of the essential oil from *Salviae folia*, in the conditions of Timişoara (2009), was in the Common sage plants cultivated over the direction E-W, followed by 21.14% content of α-thujone, of the essential Common sage oil cultivated over the direction N-S (Figure 3).

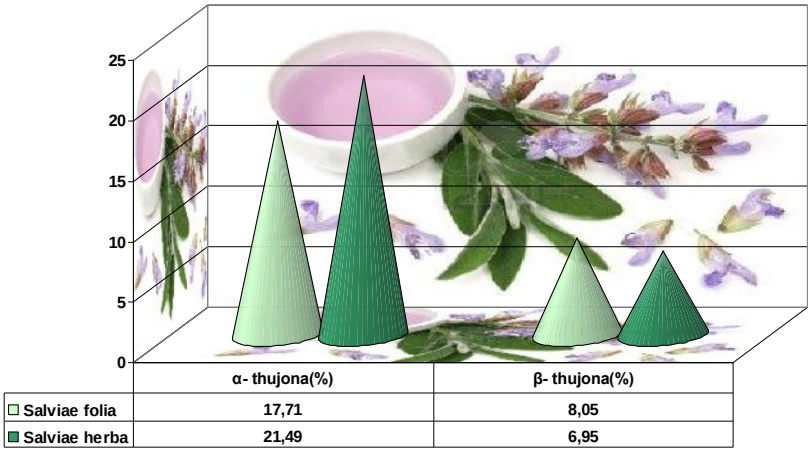


Figure 3. Variation of the content of oxygenated monoterpenes α- and β-thujone in *Salviae herba* and *Salvia folia* depending on plant row orientation (E-W)

In the Common sage plants cultivated over the direction E-W, the variation of the content of α-thujone in *Aetheroleum Salviae* differs from herba to folia. Thus, the lowest content of α-thujone, i.e. 17.70%, is in *Salviae Folia*, followed by *Salviae Herba* (21.49%) (Figure 4).

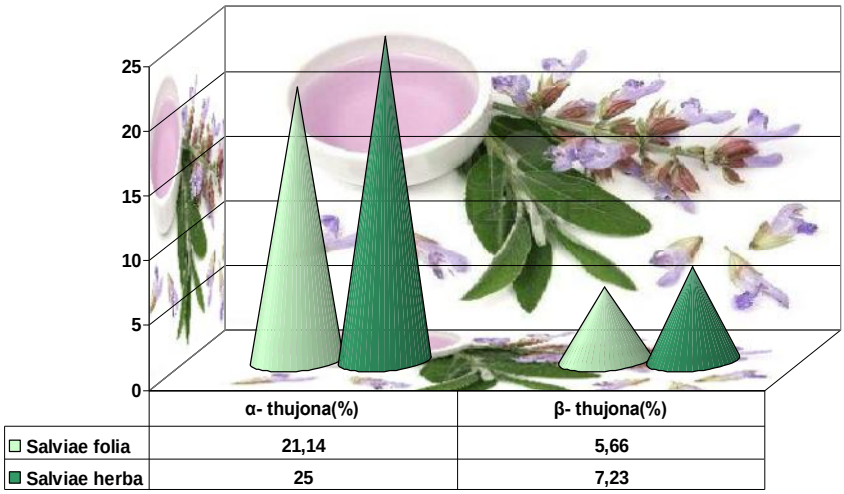
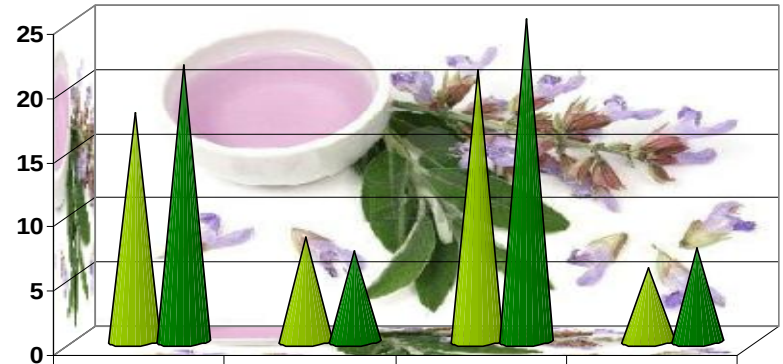


Figure 4. Variation of the content of oxygenated monoterpenes α- and β-thujone in *Salviae herba* and *Salvia folia* depending on plant row orientation (N-S)

Over the direction N-S, the variation of the content of α-thujone in *Aetheroleum Salviae* is as follows: the lowest content of α-thujone, i.e. 21.14%, is in *Salviae Folia*, followed by *Salviae Herba* (25.00%).

To note that in the meteorological conditions of the year 2009 in Timişoara, the variation of the content of α-thujone is the lowest, 17.71% in the Common sage crop cultivated over the direction E-W and when analysing biochemically the essential oil from the crude vegetal material, *folia*. The following low percentage of α-thujone of the content of essential Common sage oil was in the Common sage plants cultivated over the direction N-S, again from the crude vegetal material, *folia*: 21.14% (Figure 5).



	α- thujona(%)	β- thujona(%)	α- thujona(%)	β- thujona(%)
	E/V		N/S	
Folia	17,71	8,05	21,14	5,66
Herba	21,49	6,95	25	7,23

Figure 5 Variation of the content of oxygenated monoterpenes α- and β-thujone in Common sage depending on vegetal material and on plant row orientation (E-W and N-S, respectively)

CONCLUSIONS

Analysis of dried vegetal material of *Salviae folia* and *Salviae herba* made at the S.C. Laboratoarele Fares Bio Vital S.R.L. in Orăştie (Hunedoara County), with a gaschromatographer pointed out the following features of the content of α-thujone and β-thujone:

- the lowest content of α-thujone, 17.71%, was that of *Salviae folia*, in Common sage plants cultivated over the direction E-W, followed by *Salviae folia*, 21.49%, in Common sage plants cultivated over the direction E-W;
- the lowest content of β-thujone, 5.66%, was that of *Salviae folia*, in Common sage plants cultivated over the direction N-S, followed by *Salviae herba*, 6.95%, in Common sage plants cultivated over the direction E-W;
- the content of α-thujone, 17.71%, in *Salviae folia*, is considered particularly good and in accordance with the Standard ISO, 1997 (18.00-43.00% α- thujona and 3.00-8.50% β-thujona) concerning the limits of the content of α-thujone and β-thujone;
- the value of the chemical constituents, mainly α-thujone and β-thujone, obtained through the gaschromatography of the essential Common sage oil from the area makes possible the successful cultivation of the Common sage cultivar *De Răsmireşti* (*Salvia officinalis* L.) to obtain a good crude material.

PRACTICAL IMPLICATIONS OF THE RESEARCH

Depending on the type of product desired (*Folia, Herba, Aethoroleum*), on the different type of company – pharmaceutical of food – we can get a higher or lower content of α-thujone through different cultivation technologies of Common sage, which could capture the attention of commercial manufacturers.

With the world economic crisis, the handling of genetic material is very costly and so is the development of new cultivars that meet certain high quality requirements; it is also a very work- and time-consuming activity. But by applying proper cultivation technologies, we can spare human effort and money at the same time!

The Common sage cultivar *De Răsmireşti* (*Salvia officinalis* L.) cultivated in the Western Plain is extremely valuable since it can be cultivated on salty soils that are not suitable for other crops. These results can be of use for units processing medicinal plants in Western Romania.

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