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THE EFFECTS OF SOME PHYSIOLOGICAL AND BIOMETRIC PARAMETERS ON THE ESSENTIAL OIL CONTENT OF TWO SPECIES FROM *LAMIACEAE* FAMILY CULTIVATED IN ECOLOGICAL SYSTEM

Constantin Lungoci, Sabina-Ioana Lefter, Andreea-Nicoleta Nazare, Teodor Robu, Creola Brezeanu

***Key words**: Satureja hortensis, Ocimum basilicum, leaf area, content pigments, yield*

INTRODUCTION

Plant extracts have been employed by humans for thousands of years in traditional medicine. The *Lamiaceae* family contains important aromatic plants used in traditional and modern medicine and in the food and pharmaceutical industries. Essential oils are mixtures of volatile compounds obtained, mainly by steam distillation, from medicinal and aromatic plants. They are an alternative to synthetic additives for the food industry, and they have gained attention as potential sources for natural food preservatives due to the growing interest in the development of safe, effective, natural food preservation. *Lamiaceae* is one of the most important families in the production of essential oils with antioxidants and antimicrobial properties. (Nieto, 2017)

Many species of medicinal and aromatic plants (MAPs) are cultivated for such industrial uses, but most are still wild collected. The need for renewable sources of industrial products as well as the need to protect plant biodiversity creates an opportunity for farmers to produce such crops. The production of plants as raw material for fine chemicals is different than cultivation of ornamental or food crops. (Lubbe, Verpoorte 2011)

Essential oils exhibit two main characteristics: low risk of resistance by pathogenic microorganisms and low toxicity for humans (Daferera, 2000)

The demand for essential oils from medicinal plants has increased in recent years. (Nieto, 2017) and the ecological cultivation of these species represents a demand worthy to realize, especially because of the direct use in nutrition (spice, fresh state), or in various pharmaceutical products as (tea, tinctures, etc.).

Consumption of herbal medicines is widespread and increasing. Harvesting from the wild, the main source of raw material, is causing loss of genetic diversity and habitat destruction. Domestic cultivation is a viable alternative and offers the opportunity to overcome the problems that are inherent in herbal extracts: misidentification, genetic and phenotypic variability, extract variability and instability, toxic components and contaminants. (Peter H, 2005)

The ecological cultivation of medicinal and aromatic plants is desirable to be as widespread as possible for a better identification and exploitation of the multiple therapeutic properties that species possess, and which have biological complex of active substances. (Barbu, 2017, Robu T. and Milică, 2004)

The importance of their cultivation must be appreciated by their specific value because their properties usually cannot be replaced.

After numerous researches it has been found that medicinal and aromatic plants have a much stronger antioxidant action than certain vegetables and fruits, considered to be the most powerful antioxidant agents. (Jităreanu G., 2006)

The studied species are used for multiple purposes, including medical purposes (indigestion and digestive, gastrointestinal disorders), and in the food industry as flavor and spice (Robu T. and Milică, 2004).

Basil (*Ocimum basilicum*), belonging to the Lamiaceae family, is one of the most popular plants grown extensively in many continents around the world, especially in Asia, Europe and North America. Basil is believed to have originated in Iran and/or India. At least 150 species of the genus *Ocimum* are widely cultivated in other countries of Asia (Paton, 1992). Although several basil species are found in many regions, the species *O. basilicum* is the most cultivated variety in the world (Pripdeevech, 2010). *Ocimum* is widely cultivated and extensively used for food, perfumery, cosmetics, pesticides, medicine, and traditional rituals because of their natural aroma and flavor and other properties (Albuquerque, 1996 and Darrah, 1974).

The medicinal plants of the genus *Satureja*, commonly used herbs and shrubs, have been localized in the area of the Mediterranean region to Europe, Middle East, West Asia, North America and Africa. As annual or perennial semi-bushy, these plants inhabit arid, stony, sunny and rocky habitats along the coast of the Adriatic Sea (El-Hagrassi, 2018, Namayandeh, 2017, Jafar, 2016). *Satureja* species have been traditionally used in the treatment of various diseases such as nausea, indigestion, cramps, diarrhoea, infectious diseases and muscle pains (Jafar, 2016, Bezić, 2009)

MATERIAL AND METHOD

The experimental field was established in the Collection of Medicinal and Aromatic Plants within the framework of USAMV Iaşi. The predominant soil was chernozem, lutus, formed on leoxoid clays, having the chemical properties: hygroscopicity index between 7.01 and 8.29 and a C / N ratio <15 in the upper horizon, organic matter 3-4.1%, N 0.17-1.94%, available P_2O_5 38 mg / kg, available K_2O 143-181 mg / kg, pH 7-7.3 (Onofrei, 2017).

The establishment of the culture was carried out by seedlings for both species. The distance between rows was 70 cm and between plants in a row 35 cm in the case of *Satureja hortensis* L. species and 70 cm between rows and 28 cm in the case of *Ocimum basilicum* L. species (all varieties).

Satureja hortensis L. species with one variety (Daria) and *Ocimum basilicum* L. with three varieties (Lemon, Vert and Alimentary) are the biological material that the studies were undertaken.

During the vegetation period until the blooming time, the main biometric determinations were made, recording the number of days between the main phenophases. The leaf surface is a very important parameter, knowing that the degree of accumulation of the active principles depends on this element. The determinations were made with the AM 350 Area Meter apparatus.

The Opti-Sciences CCM-200 was used to determine the content in chlorophyll pigments.

The extraction of the volatile oil was performed using the hydrodistillation method (widely used in specialized laboratories). The oils were isolated from herba using a modified Clevenger – type apparatus (Mechkovski and Akerele, 1992). The isolated oils were dried over the anhydrous sodium sulfate (Merck, Germany). The samples were stored in a refrigerator for future investigations as Gas Chromatography (GC) and Gas Chromatography / Mass Spectrometry (GC/MS) analysis. The process by which the water vapor passes through the vegetal material, in this case of herba, after which by condensation and separation, the volatile oil is obtained, the process takes 2.5 h.

The yield on drying, a particularly important parameter to be known especially in the supplies of fresh herba and the estimation of losses through drying or the evaluation of the production obtained from the field. Statistical analysis of data obtained from biometric measurements and determinations was performed by analysis of variance (ANOVA). The obtained results were processed, calculating the limit differences for the probability of transgression of 5%, 1% and 0.1%. (Jitareanu, 2016).

RESULTS AND DISSCUSION

In the case of *Satureja hortensis* L., the number of days from planting to the beginning of flowering was about 48 days and until the maximum flowering, the time to harvest was 58 days. The plants had a small size (on average 30 cm), with a variable number of branches (on average 17.6). The plants had an average weight of 129.6 grams, and the volatile oil resulting from one kilogram of fresh herba was 6.76 ml (0.676%). The yield on drying was about 1:3. The data are shown in table 1.

Table 1 Biometric determinations at *Satureja hortensis* L.

	Number of branches	Weight (g)	Essential oil content (%)	Drying yield (%)
Satureja hortensis	17.7	388.2	6.76	1 : 2.97

The establishment of basil culture was done by seedlings, in the last decade of May. The number of days from planting to the appearance of the first flowers at the base of the inflorescence was about 28 days, and until flowering 50% of the plants was 18 days from the beginning of flowering. The investigations were made at 50% flowering (Figure 1, Table 2).

Following the measurements with the Opti-Sciences CCM-200 mobile device, it can be seen that in the upper leaves there is the highest quantity of chlorophyll pigments, with values between 19.37 for the Lemon variety, 17.13 for the Vert variety and 12.47 for the Alimentary variety. Also, the content of chlorophyll pigments is decreasing from the upper level of the foliar apparatus to the lower ones, being 12.03 for the Lemon variety, 9.27 for the Vert variety and 9.33 for the Alimentary variety. Data are presented in table 1.

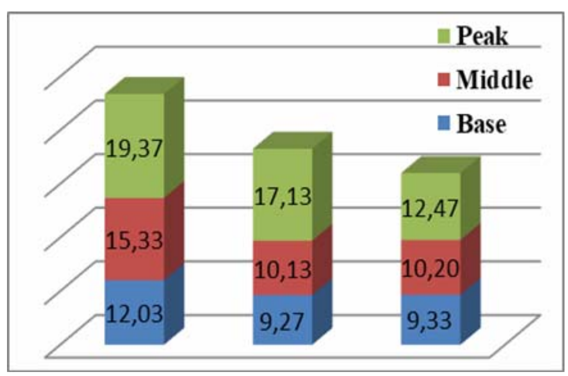

Fig. 1 Variation of in chlorophyll content depending on leaves position on plant at three basil varieties

From a physiological perspective, leaf chlorophyll content is, therefore, a parameter of significant interest. As a general observation in case of basil, during storage leave senescence was similar to darkening tissues and also with a decrease of volatile oil content.

Considering that senescence has a direct relationship with on leaf chlorophyll content, it is important with basil producers and consumers to know the chlorophyll content before and after harvest. (Francisco, 2010, Table 2 and 3).

Table 2. Chlorophyll content in basil varieties

Variant	Chlorophyll content	Differences	
		%	ml/kg
Lemon	46.7	121.61	8.3
Vert	36.5	105.26	-1.9
Alimentary	32.0	126.32	-6.4
Mean	38.4	100	0

The most of its bioactive components of basil in the leaf, where it is stored in secretory bags. Therefore, the knowledge of the leaf surface is an important factor from two points of view: it increases the quantity of raw material produced and used in food or industrialization and presents a greater capacity for synthesis of volatile oil.

In figure 2 and table 2 it can be seen that, the largest leaf surface is present in the Alimentary variety with a value of 10,043 mm^2 on average per plant, followed by the Lemon variety with an area of 5,745 mm^2 and the Vert variety with a leaf surface of 2,969.7 mm^2.

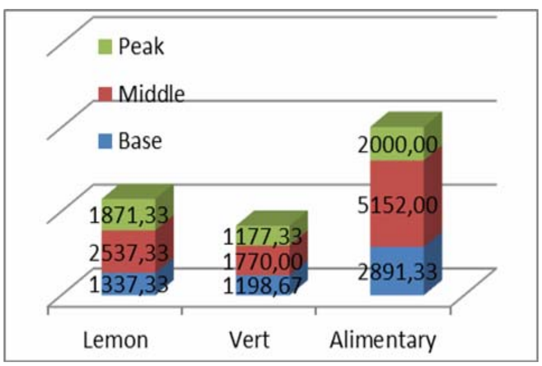

Fig. 2. Leaf area on different levels (mm^2)

Regarding the foliage arrangement for all three varieties, it is located in the middle part of the plants, the values being 5152 mm^2 at Alimentary variety, 2537 mm^2 at Lemon variety and 1770 mm^2 at Vert variety.

The number of branches shown in figure 3 and table 3 is an important trait because determines the size and arrangement of the bush, can influence the distance between plants on a row, but also the distance between rows.

This parameter is variable from one variety to another and has an influence on the yield of volatile oil and also on the herba.

Table 3. Leaf area at basil

Variant	Leaf surface	Differences	
		%	mm^2
Lemon	5746	86.47	-899.1
Vert	4146	62.39	-2499.1
Alimentary	10043.3	151.14	3398.2
Average	6645.1	100	0

In case of investigated varieties, on average, the big number of branches was found at the Lemon variety: 16.67 branches per plant, followed by Vert with 12 branches and the Alimentary variety with 10.33 branches (figure 3, table 4)

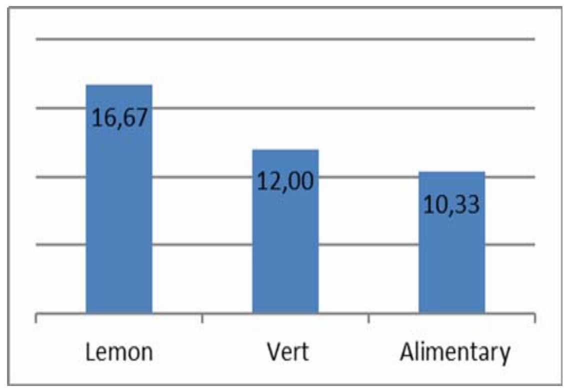

Fig. 3. Number of branches per plant

Table 4. Number of branches per plant

Variant	Ramification number	Differences	
		%	No of pl
Lemon	16.67	128.46	3.7
Vert	12	92.31	-1
Alimentary	10.33	79.23	-2.7
Average	13	100	0

The weight of plants is an important factor in yield estimation. Knowing the density and the average weight of plants of each crop, the yield is easily estimated. This was also done in the study conditions.

In the research conditions, the highest average weight of the plants was realized at the Lemon variety with a value of 294 grams, followed closely by Vert variety with 247.3 grams per plant and the lowest value was registered at the Alimentary variety 103.23 grams figure 4. In case of the Alimentary variety, the difference is significant and negative. The other varieties are not statistically assured (tab. 5). The results reflect the existing data in the specialized literature.

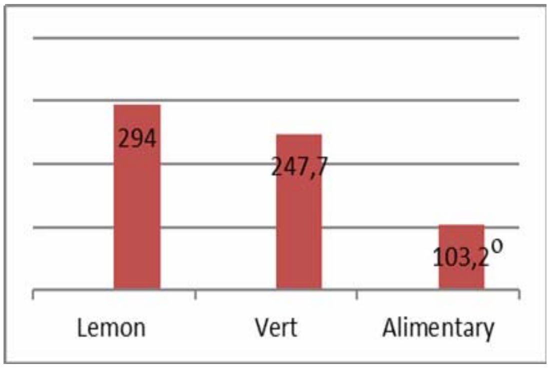

Fig. 4 . Plant weight of *Ocimum basilicum* L

Table 5. Plants weight of *Ocimum basilicum* L.

Variant	Weight herba	Differences	
		%	No./pl.
Lemon	294	136.74	79
Vert	247.7	115.21	32.7
Alimentary	103.2	48	-111.8
Average	215	100	0

All the parameters determined are correlated with volatile oil content. The amount of essential oil is an element that varies according to many factors, such as: variety, organ, climatic conditions, harvesting phenophase, time of harvesting, etc. In this case, the highest content of volatile oil was determined at the Alimentary variety and can be related with the large leaf surface. It recorded a value of 2.4 ml / kg fresh herba, harvested at 2 p.m.

For the Vert variety it was 2 ml / kg fresh herba and 1.3 ml / kg in the case of Lemon variety. According to the statistical analysis, the results were positively significant in case of Alimentary variety and negatively significant for Lemon variety. The interpreted results are shown in figure 5 and table 6.

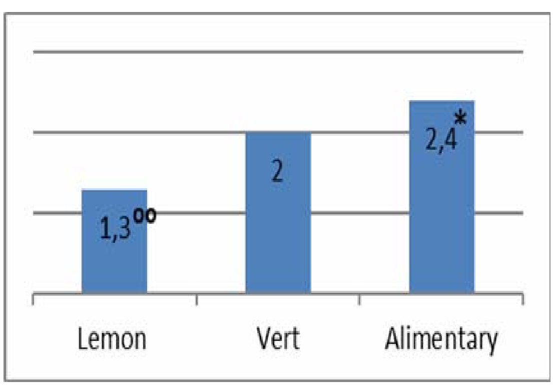

Fig. 5. The content of volatile oil

Table 6. The content of volatile oil

Variant	Essential oils	Differences	
		%	ml/kg
Lemon	1.3	68.42	-0.6
Vert	2	105.26	0.1
Alimentary	2.4	126.32	0.5
Average	1.9	100	0

CONCLUSIONS

The ecological culture of medicinal plants represents an area of interest for the future. The results of this study showed that in case of Alimentary var., with the highest essential oil content, is strongly influenced by the leaf area.

Although the other varieties showed high values on some parameters (the number of branches, the weight of the plants and the content in chlorophyll pigments), a large leaf surface leads to the synthesis and accumulation of a high amount of volatile oil, which is rich in bioactive principles.

The determination species capacity to occur significant volatile oil content, potentially useful encourages the extensive study.

ABSTRACT

The cultivation of medicinal plants in the ecological system is a topical topic, with an increasing emphasis.

The society is aware that products of plant origin that are not subjected to an industrialization process and are used directly in food and therapy should be free of chemical residues (chemical fertilizers, pesticides).

The two plants studied are important for the aromatic and spicy role they have in the food alimentation, but also for the antiseptic capacity given by linalool (basil) and carvacrol (thyme). The study synthesizes a series of biometric and physiological determinations that highlight the productivity of herba mass and volatile oil.

Planting was done at a distance of 70 cm between rows and 35 cm respectively 28 plants between rows. Harvesting of plants for the determination was done in full bloom.

The obtained results show that for the variety with the highest foliar surface the volatile oil content is significant, of 2.4 ml / kg, and the highest quantity of herba was obtained for the variety with the most branches.

The drying efficiency was 1: 2.1 for Lemon and Vert and 1: 1.7.

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REFERENCES

1. ALBUQUERQUE, U.P., 1996, Taxonomia e ethnobotânica *Ocimum basilicum* L. (*Lamiaceae*) – renfêrencia especial para Pernambuco Recife, Dissertação (Mestrado em Biologia Vegetal), Centro de Ciencias Biologicas, Universida de Federal de Pernambuco, , p 125
2. BARBU I., 2017 - Cercetări cu privire la elaborarea tehnologiei de cultură, în sistem de agricultură biologică, a unor specii de plante aromatice şi condimentară. Teză de doctorat
3. BEZIĆ, I. SAMANIĆ, V. DUNKIĆ, V. BESENDORFER, J. Puizina, Molecules, 2009, 14, 925.
4. DAFERERA D.J., ZIOGAS B.N., POLISSIOU M.G. GC-MS analysis of essential oils from some greek aromatic plants and their fungitoxicity on *Penicillium digitatum*. J. Agric. Food Chem. 2000;48:2576–2581. doi: 10.1021/jf990835x. [PubMed] [CrossRef] [Google Scholar]
5. DARRAH, H., Investigation on cultivars of Basils (Ocimum), Economic Botany 1974, 28, 63-67, doi: 10.1007/BF02861381
6. FRANCISCO H. RUIZ-ESPINOZA; BERNARDO MURILLO-AMADOR; et al., 2010 Field evaluation of the relationship between chlorophyll content in basil leaves and a portable chlorophyll meter (SPAD-502) READINGS in Journal of Plant Nutrition http://www.informa world.com/smpp/title~content=t713597277
7. HAGRASSI, M. El, W. E. ABDALLAH, A. F. OSMAN, K. A. ABDELSHAFEEK, 2018, Asian Journal of Pharmaceutical and Clinical Research, 11, 142
8. JAFARI, F. GHAVIDEL, M. M. ZARSHENAS, 2016, Journal of Acupuncture and Meridian Studies, 9, 118.
9. JITĂREANU G., 2006 – Metode moderne de cercetare, curs. Ed. „Ion Ionescu de la Brad", Iaşi;
10. LUBBE A., VERPOORTE R., 2011, Cultivation of medicinal and aromatic plants for specialty industrial materials, Industrial Crops and Products, Volume 34, Issue 1, Pages 787, ISSN 0926-6690, https://doi.org/10.1016/j.indcrop.2011.01.019
11. MECHKOVSKI, A.; AKERELE, C. O., 1992, Quality control methods for medicinal plant materials. Who/Pharm/92.559. Switzerland: World Health Organization.
12. NAMAYANDEH, N. MOKHTARI, M. M. KAMELMANESH, M. T. Bedaf, 2017, Journal of Genetics, , 96, e5.
13. NIETO G. Biological Activities of Three Essential Oils of the Lamiaceae Family. *Medicines (Basel)*. 2017; 4(3):63. Published 2017 Aug 23. doi:10.3390/medicines4030063
14. ONOFREI V., 2017 - Cercetări privind influența unor fertilizanți foliari ecologici asupra producției şi calității acesteia la unele specii medicinale şi aromatice. Teză de doctorat.
15. PATON, A., 1992, A synopsis of Ocimum L. (Labiatae), Kew Bulletin, 47, 403-436
16. PETER H. CANTER, HOWARD THOMAS, EDZARD ERNST, 2005, Bringing medicinal plants into cultivation: opportunities and challenges for biotechnology, Trends in Biotechnology, Volume 23, Issue 4, , Page 183, ISSN 0167-7799, https://doi.org/10.1016 /j.tibtech.2005.02.002.
17. PRIPDEEVECH, P.; CHUMPOLSRI, W.; SUTTIARPORN, P.; WONGPORNCHAI, S., 2010, The chemical composition and antioxidant activities of basil from Thailand using retention indices and comprehensive two dimensional gas chromatography, Journal of the Serbian Chemical Society, 75(11) 1503-1513, doi: 10.2298/JSC100203125P
18. ROBU T., MILICĂ C., 2004 – Plante medicinale autohtone. Ed. INSTITUTUL EUROPEAN, Iaşi;

*** www.gazetadeagricultura.info.

AUTHORS' ADDRESS

LUNGOCI CONSTANTIN, LEFTER SABINA, NAZARE ANDREEA, ROBU TEODOR - "Ion Ionescu de la Brad" University of Agricultural Sciences and Veterinary Medicine, Street, no. 3, Iaşi, e-mail:constantinlungoci@uaiasi.ro; teorobu@uaiasi.ro

BREZEANU CREOLA - Vegetable Research and Development Station, Calea Barladului, Street, no. 220, Bacau, Romania, e-mail: creola.brezeanu@yahoo.com.