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MICROMORPHOLOGICAL TRAITS AND BIOCHEMICAL PATTERN OF *PEROVSKIA ATRIPLICIFOLIA* BENTH., PLANT WITH PHYTOREMEDIATIVE POTENTIAL

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Abstract. The deciduous semi-woody shrub *Perovskia atriplicifolia* Benth. (*Lamiaceae*) is native in the rocky areas of Pakistan, Afganistan, Iran, and Tibet and it is mainly known for the culinary, flavoring and ornamental qualities. The essential oils have pharmacological effects based on the antimicrobial and antimutagenic activity of their constituents. The plant is also cultivated in Europe and recently it was experimentally introduced in Romania, in order to exploit its medicinal valences (based on aerosol properties), the potential as remediative plant and its decorative traits. In this context, a deeper knowledge of the morphology and phytochemical profile of this newly cultivated plant in Romania becomes necessary, knowing that, besides the genetic factors, the environmental conditions and the geographical variation are factors highly influencing the plant morphological and biochemical phenotype. The objective of this approach is, therefore, to analyze some morphological traits and the distribution rate of the volatile oil–producing secretory trichomes on the surface of the aerial vegetative organs (stems and leaves) and reproductive organs (flowers) and to establish the chemical phenotype by analyzing the composition of the volatile oils produced by these organs in anthesis stage. The micromorphological investigations have been carried out using a surface electron microscopy, at a TESCAN VEGA II SBH microscope. The volatile oils extraction was conducted using a Clevenger hydrodistillation system, and the component separation was performed by gas chromatography, with a 6890 Agilent GC/MS. The volatile compound identification was made using the NIST spectral bank and Kovats indexes. In our biological material, grown in the pedoclimatic conditions of Moldova region, the secretory trichomes are multicellular structures, with a specific conformation, in relation to the analyzed plant part. The pattern of volatile oils is variable and the number of constituents depends on the investigated organ. The main compounds of the volatile oil from *P. atriplicifolia* are the monoterpenes which confer to this plant the role in improving of air quality.

Key words: micromorphology, secretory trichomes, volatile oils, vegetative organs, flowers, anthesis stage

1. INTRODUCTION

Perovskia atriplicifolia (Russian sage) is native to Central Asia, in an area including Afghanistan, Iran, Pakistan, and Tibet. In Romania, this plant is cultivated in the Botanical Gardens from Bucharest, Cluj, Targu Mures and Iasi, also in the Botanical Park from Gurahont. *P. atriplicifolia* is known as a medicinal plant, used in traditional medicine in Central Asia as remedy in lung congestion such as bronchitis, while in Russia its leaves are smoked as euphoriant. In the West, it is used almost exclusively as ornamental plant in planning of parks and gardens. Besides medicinal properties, the species has obvious attributes that recommend it for the use in phytoremediation (Djenbaev et al., 2006).

Environmental pollution with different mineral substances and heavy metals is a global problem (Tomaškin, 2007; Secu et al., 2008; Lacatusu & Lacatusu, 2008); the development of new phytoremediation technologies based on the use of plants in cleaning up of the contaminated soils is of large interest (Füleky & Barna, 2008). Although the number of plants used in phytoremediation is already very high, finding new species with these capabilities is of great importance because of the difficult environmental conditions in the areas requiring remediation. At least 45 families, including *Lamiaceae*, have been identified to hyperaccumulate heavy metals (Jadia & Fulekar, 2009, Pandey et al., 2007, Reeves & Baker, 2000, Scora & Chang, 1997). These plants appear to be a good choice for phytoremediation since recent studies proved that heavy metals accumulated by these plants do not appear in the essential oil and that some of these species are able to grow in metal contaminated sites without significant yield reduction (Sharma et al., 2009).

Concerning the possibility to use *P. atriplicifolia* in phytoremediation, Djenbaev et al., 2006 noted that in uranium radioactively contaminated areas of Kadji-Sai province (Kyrgyz Republic), the individuals belonging to some species (among them – *P. atriplicifolia*) showed an intensive growth. For this reason, these plants can be considered as metal hyper accumulators because of their potential to accumulate and tolerate high amounts of heavy metals from contaminated soils. Therefore, they can be used to clean up the respective soils. These data are sustained by our preliminary investigations (unpublished data) on *P. atriplicifolia* behaviour in the treatments with varying concentrations of cadmium chloride and lead carbonate.

P. atriplicifolia is drought-tolerant. All parts of *P. atriplicifolia*, including the flower heads, have glandular and nonglandular silvery hairs (Rao, 1926). Their presence in high number confers to the plants an increased resistance to pests and drought, leading to the possibility to maintain them in culture without special efforts. Being native in harsh environments, this species is highly resistant to urban pollution (Djenbaev et al., 2006). Additionally, because of the low tolerance to excess water, *P. atriplicifolia* could be used for restoration of arid areas.

The high rates of growth, good germination, adaptation to drought, low need for cultivation procedures, resistance to metals and radioactivity are factors recommending *P. atriplicifolia* to be used used in phytoremediation.

The structure and function of trichomes from different species of the *Lamiaceae* family are well documented (Ascensão & Pais, 1998, Bosabalidis, 1990, Corsi & Bottega, 1999, Combrinck et al., 2007, Gairola et al., 2009, Marin et al., 2006; Werker, 1993, Zamfirache et al., 2009).

The aim of this paper was to determine the morphological traits and distribution of secretory hairs (trichomes) in *P. atriplicifolia* Benth. cultivated in North-Eastern zone of Romania and to perform a qualitative and quantitative analysis of volatile oils produced by these structures from different vegetative and reproductive plant organs in order to a better understanding of the expression of some specific environmental conditions into the morphological and biochemical phenotype.

2. MATERIAL AND METHODS

The flowering plant material was collected from Iaşi area. A voucher specimen is stored in the Herbarium of Faculty of Biology, "Al. I. Cuza" University from Iaşi, Romania.

For scanning electron microscopy (SEM) investigations (performed in the Plant Morphology and Anatomy Laboratory of the Faculty of Biology, "Al. I. Cuza" University from Iaşi), the investigated material consisted in small pieces from leaves, stem and floral pieces. The samples were noted as follows, considering their length: leaf 1 (5 mm), leaf 2 (1 cm), leaf 3 (1.5 cm), leaf 4 (2.5 cm). The plant material was fixed in FEA (formol: 70% ethanol: acetic acid –5:90:5) for 48 hours, washed with distilled water and stored in 70% ethanol. Some pieces were sectioned under the stereomicroscope with a razor blade. After dehydration in a graded ethanol series (80%, 90%, and 100%) and acetone, the material was critical point dried with CO_2 (using a EMS 850 Critical Point Dryer), sputter-coated with a thin layer of gold (30 nm) (using a EMS 550X Sputter Coater) and, finally, examined by scanning electron microscopy (Tescan Vega II SBH) at an acceleration voltage of 27.88 kV.

The measurements of the glandular hairs (diameter and density) were made using the biometrical software from Nikon (NIR-Demonstration). Fifty measurements were made for glandular hair diameter and ten measurements for hair density.

The volatile oil extraction was conducted using a Clevenger hydrodistillation system. The component separation was performed by gas chromatography using a 6890 Agilent GC-MS. The volatile compound identification was made using the NIST spectral bank and Kovats indexes, at the Faculty of Horticulture, U.S.A.M.V. Bucharest.

3. RESULTS AND DISCUSSIONS

The leaves, stems and flower pieces of *P. atriplicifolia* have small and large peltate glandular hairs, rare capitate hairs, as well as multicellular ramified nonglandular hairs.

The density of glandular trichomes in different leaves was variable on all investigated organs; the nonglandular hairs (trichomes) formed a dense covering, obscuring the epidermal surfaces.

The density of the glandular hairs constantly decreases from young to mature leaves (Table 1); this feature is correlated with the continuous growth of the foliar surface and the stop of initiation of new glandular hairs early in leaf ontogenesis.

The peltate hairs were quite uniform in morphology, but we can distinguish two types of this hair category. Type I – most widely distributed, both on leaves and on stems and floral parts, had a short unicellular stalk and a large secretory head – with 12 glandular cells. This type of hairs is widespread on all vegetative and reproductive organs (Fig. 1). They are noticed also on the ventral part of the stamens (Fig. 2-A). The occurrence of the glandular hairs on the reproductive organs of some *Lamiaceae* species has been reported by Werker et al., 1985 and Ascensão & Pais, 1998. Their function is still uncertain; they could have a role in pollinator attraction or in protection against predators (Ascensão & Pais, 1998). Type II – is rare and is present only on the sepal surface. It is similar with Type I except that it has a long stalk (Fig. 2-B).

The capitate trichomes (Type I) consisted of a basal cell, a short unicellular stalk and bi-cellular secretory head (Fig. 1-D; Fig. 3-C, D). Type II – is unfrequent - we found it only on stem surface. It has a basal part similar to the normal branched tector hairs and one terminal part similar to that of Type I (Fig. 2-C, D). This rare type of double-function trichomes was mentioned for the first time in 2007 by Nikolakaki & Christodoulakis on the leaves of *Phlomis fruticosa*. They considered this kind of hair to be unique among Mediterranean plants. Our investigations demonstrate the presence of this hair type on *P. atriplicifolia* stem.

The activity of medicinal plants can be attributed to their secondary metabolites, some of which occur in the essential oil of aromatic plants (Combrinck et al., 2007). The electron microscopy investigations regarding the cuticle which covers the glandular hairs did not reveal any pores or spaces through which the volatile oils could exude.

The secreted substances was not released until the cuticle broke, or at the end of the life of the glandular hair (Fig. 3-A, B). The distribution and structure of trichomes on plant surfaces have an important role on the control of transpiration and temperature of the organ on which they occur (Bosabalidis, 2002). In addition, it has been postulated that the trichomes density and phenolic compounds formed in these structures afford the organ protection against UV-B radiation, which results from exposure to sunlight (Liakoura et al., 1997).

The presence, yield and composition of secondary metabolites in plants, thus the components in volatile oils too can be affected in a number of ways by several factors that include: physiological variations, environmental conditions, geographic variations, genetic factors and evolution etc. (Figueiredo et al., 2008).

In a classical study, using vacuum distillation and chemical tests, Rao (1926) found α-pinene, β-pinene, camphene, borneol, bornyl acetate, α-caryophyllene and aromadendrene in the volatile oil of *P. atriplicifolia*. Later studies on the volatile oil of *P. atriplicifolia* from Afghanistan confirmed Rao's results and found, in addition, α-terpinene, 1,8-cineole, camphor, menthol and cedrol, but not aromadendrene (Younos et al., 1972).

Table 1 – Glandular hairs density and diameters variation on different leaves at *Perovskia atriplicifolia* Benth. (values represent means ± standard deviations)

Plant part	Large hairs density (per mm^2)		Small hairs density (per mm^2)		Large hairs diameter (µm)		Small hairs diameter (µm)	
	Lower epidermis	Upper epidermis	Lower epidermis	Upper epidermis	Lower epidermis	Upper epidermis	Lower epidermis	Upper epidermis
Leaf 1	52.65±6.54	53.57±7.07	58.98±8.32	56.12±7.58	71.51±10.64	62.16±6.97	15.36±2.0	20.31±2.15
Leaf 2	44.11±7.91	47.23±6.32	57.66±7.45	50.29±9.12	61.24±5.65	65.85±6.5	15.53±1.32	20.67±1.98
Leaf 3	42.87±5.74	42.13±7.81	56.39±6.51	47.08±6.34	61.78±5.58	64.22±5.45	16.86±1.77	20.45±2.37
Leaf 4	40.24±5.62	40.56±5.44	42.31±7.32	44.62±6.81	61.94±7.59	64.97±5.06	17.73±2.47	20.58±2.36

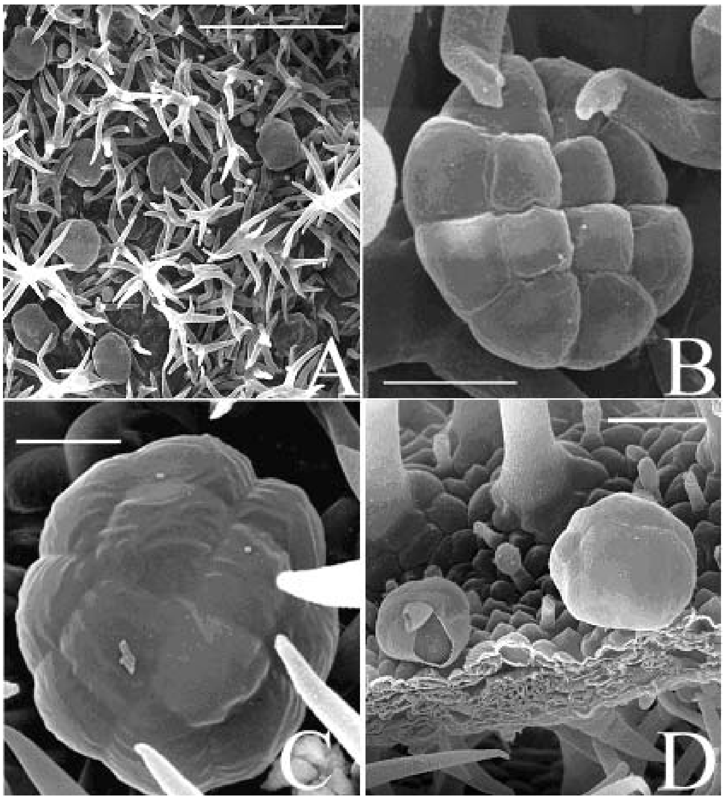

Figure 1. A - *Perovskia atriplicifolia* Benth.: General aspect of the lower epidermis of a mature leaf (leaf 4), scale bar = 200µm, B, C - Peltate multi-cellular glandular hairs on upper epidermis (Type I): B - young leaf (leaf 2), C - mature leaf (leaf 4), scale bar = 20µm, D - Peltate (Type I) and capitate glandular hairs on a mature sepal, scale bar = 50µm

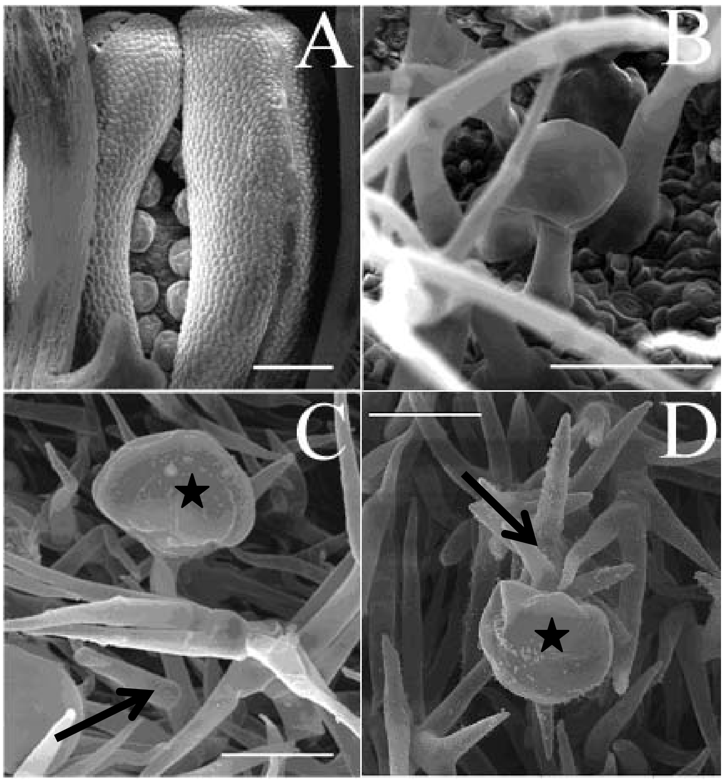

Figure 2. *Perovskia atriplicifolia* Benth.: Peltate glandular hairs (Type I) on the anther, B - Peltate glandular hairs (Type II) on the stem, C, D - capitate glandular hairs (Type II) – branched stalk (arrow) and secretory head (star) could be observed, scale bar = 50 µm

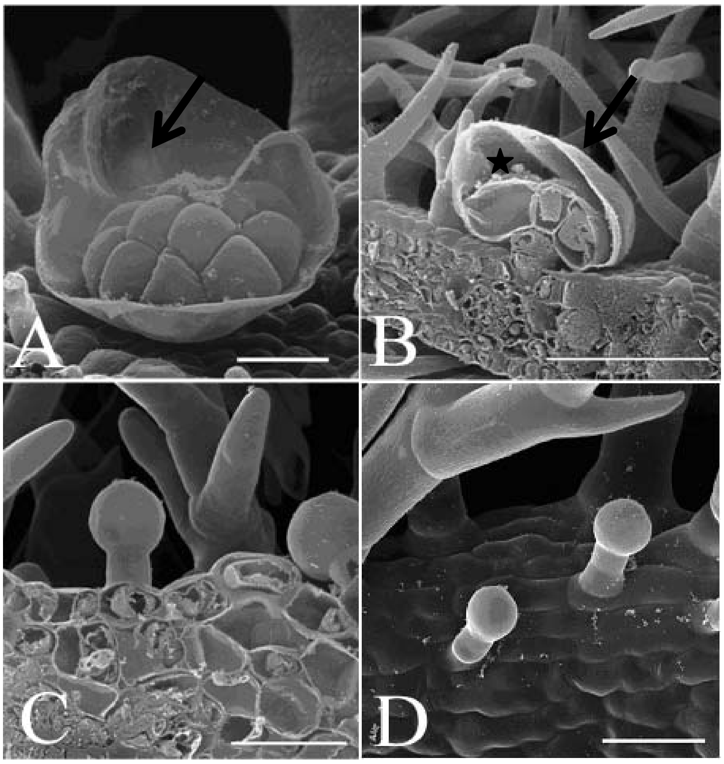

Figure 3. A - *Perovskia atriplicifolia* Benth.: Peltate glandular hair (Type I) on a sepal – the cuticle (black arrow) was removed from the secretory cells, scale bar = 20µm, B - cross section through a petal and longitudinal section through a peltate glandular hair (Type I) – the cuticle (white arrow) and the large subcuticular space (star) could be observed, C - Capitate hair (Type I) on a very young leaf (leaf 1), D - Capitate hair (Type I) on a young sepal, scale bar = 20 µm

In the essential oil obtained from the glandular trichomes of *P. atriplicifolia* cultivated in Italy, camphor (14.9%) was the main constituent (Mucciarelli et al., 1993). More recently, the essential oil of *P. atriplicifolia* cultivated in Iran has been described (Sefidkon et al., 1997).

Out of 19 compounds identified by Jassbi et al.(1999) in the volatile oil of *P. atriplicifolia* from Pakistan, the monoterpenes, D3-carene (22.3%) and 1,8-cineole (27.5%) amounted to 50% of the total oil. β-caryophyllene (10.8%) and α-humulene (5.7%) were the dominant sesquiterpenes.

In Romania, the first studies on chemical composition of this species have been carried out and still are at the Faculty of Biology, "Al. I. Cuza" University from Ia□i. No other similar references concerning the study of this species cultivated in Romania were found.

Our results regarding chemical composition of the volatile oil of the whole plant of *P. atriplicifolia* show that the main constituents with the highest percentage are: eucalyptol (24.62%), o-cymene (17.87%) and borneol (15.37%), followed by γ-terpinene (6.39%), bornyl acetate (4.83%) and camphene (4.66%) (Table 2). A number of 32 compounds have been identified in the volatile oil of whole plant of *P. atriplicifolia* collected from Iaşi area. 27 from these compounds have been identified in the volatile oil from stems. Also, 27 from constituents evidenced in whole plant have been found in leaves, while 24 of them have been detected in the volatile oil obtained from flowers. The volatile oil obtained from flowers displayed the highest number of chemical constituents (47). 24 of the chemical constituents of volatile oils are common to all tested plant organs, some of these being present in the highest levels – probably they confer the common aromatic fragrance to volatile oil. Eucalyptole is present in a higher percentage in leaves (19.70%) than in stems (18.44%) and flowers (16.30%). The stem is richer in o-cymene (18.23%) than leaves (13.91%) and flowers (11.81%). The monoterpene γ –terpinene reached a higher percent in flowers (4.37%), compared to stems (1.81%) and leaves (1.54%). Borneol is predominant in leaves (20.41%), comparatively to stems and flowers (approximately 9%).

Monoterpenes are emitted by forests and form aerosols that can serve as cloud condensation nuclei (CCN). Such aerosols can increase the brightness of clouds and cool the climate (Spracklen et al., 2008). The main constituents of the volatile oil of *P. atriplicifolia* investigated in this research being monoterpenes, we can conclude that this plant grown in temperate continental conditions has a great importance due to its aerosol emitting capacity. It creates a pleasant fragrant and fresh atmosphere.

Eucalyptol exerts decongestant and expectorant action (Nigg et al., 1992), borneol has antibronchitic effects (Harborne et al., 1983), and camphene shows expectorant properties (Hansel et al., 1992). Bornyl-acetat is known to have antibacterial and antiviral properties (Leung et al., 1995) and also manifests an expectorant effect (Harborne et al., 1983). These properties of the main constituents of the studied volatile oils, all of them being monoterpenes, make *P. atriplicifolia* a valuable plant in improving the quality of the air. From sesquiterpenes, β-cariophyllene and α-cariophyllene are present in all investigated plant parts, both being identified in higher amounts in leaves.

Table 2 – Comparison between the main chemical constituents identified in volatile oil of different organs of *Perovskia atriplicifolia* Benth.

No.	Chemical constituents	Whole plant (%)	Stem (%)	Leaves (%)	Flowers (%)
1	tricyclene	0.07	0.11	0.11	0.80
2	α-thujene	0.15	0.95	0.80	-
3	α-pinene	1.38	3.41	3.21	7.33
4	camphene	4.66	3.39	3.47	2.52
5	sabinene	0.27	-	-	-
6	β-pinene	3.58	3.03	2.37	2.90
7	3-octanone	0.08	-	0.26	-
8	β-myrcene	1.10	0.60	0.64	0.68
9	α-phellandrene	0.13	-	-	-
10	3-carene	0.06	-	-	-
11	α-terpinene	0.94	0.20	0.36	0.50
12	o-cymene	17.87	18.23	13.91	11.81
13	eucalyptole	24.62	18.44	19.70	16.30
14	γ-terpinene	6.39	1.81	1.54	4.37
15	terpinolene	0.26	0.13	0.13	0.17
16	linalool	0.73	0.62	0.86	0.57
17	α-thujone	0.20	0.25	0.18	-
18	cis-sabinol	0.30	0.34	0.20	0.34
19	camfor	0.12	0.54	0.40	0.25
20	borneol	15.37	9.61	20.41	9.71
21	terpinolene 4-ol	0.36	0.29	0.45	0.36
22	α-terpineole	0.39	0.33	0.56	0.47
23	bornil acetate	4.83	14.65	5.02	7.46
24	timol	0.15	0.40	0.49	0.40
25	carvacrol	0.15	0.49	0.53	0.27
26	α-terpinil acetate	1.77	2.76	2.07	1.40
27	β-cariophyllene	3.29	2.36	4.66	3.32
28	α-cariophyllene	3.04	2.85	5.11	3.24
28	aromadendrene	0.07	-	-	-
30	α-selenene	0.08	0.16	-	-
31	cariophyllene oxide	1.33	6.02	4.78	2.67
32	ledol	0.26	1.06	1.60	0.81
33	trans β-ocymene	-	0.20	-	0.42
34	izopropilmetilbiciclohexane 2-ol	-	0.32	0.37	0.52
35	bornil formiate	-	0.12	-	0.11
36	β-selinene	-	0.11	-	0.33
37	δ-cadinene	-	0.18	-	0.08
38	γ-cadinene	-	0.23	-	0.31
39	germacrene-B	-	0.27	0.15	0.98
40	trans bisabolene epoxide	-	2.70	2.91	1.41
41	azulene	-	-	0.17	-
42	carvone	-	-	0.20	9.06

No.	Chemical constituents	Whole plant (%)	Stem (%)	Leaves (%)	Flowers (%)
43	cis bisabolene epoxide	-	-	0.46	-
44	selinene 4-ol	-	-	-	0.10
45	selinadiene	-	-	-	0.64
46	germacrene D	-	-	-	0.73
47	γ-humulene	-	-	-	0.12
48	epi biciclosesquiphelandrene	-	-	-	0.10
49	cis jasmone	-	-	-	0.10
50	β-burbonene	-	-	-	0.46
51	dehidrocarvil acetate	-	-	-	0.09
52	pupegone	-	-	-	0.49
53	cis charveole	-	-	-	0.12
54	trans charveole	-	-	-	0.08
55	dehidrocharveole	-	-	-	0.49
56	menthone	-	-	-	0.49
57	β-thujone	-	-	-	0.17

4. CONCLUSIONS

The density of glandular trichomes of *Perovskia atriplicifolia* was variable on all investigated organs, the nonglandular hairs forming a dense covering, obscuring the epidermal surfaces. The density of the glandular hairs constantly decreases from young to mature leaves, correlated with the continuous growth of the foliar surface and the stop of initiation of new glandular hairs early in leaf ontogenesis.

The specific chemical composition of the volatile oils secreted by investigated specimens of *Perovskia atriplicifolia* reflects their biochemical reaction to the specific ecological conditions of the locations in which they have been cultivated. There is also an important biochemical variation, both quantitatively and qualitatively, depending on analyzed organ, possibly in relation to density of secretory hairs and the physiological state in the moment of analysis. Our results contribute to the knowledge and understanding of the type of metabolic response to some specific conditions existing in living area of the studied species.

Our results regarding the chemical composition of the volatile oil of *Perovskia atriplicifolia* show that the main constituents are monoterpenes, they conferring to the plant an aerosol emitting capacity, with positive effects in improving the quality of the environment in public places such as gardens and parks.

The structural and biochemical proprieties of *P. atriplicifolia* confirm their resistance to drought conditions; given its resistance to drought and native resistance to pollution, this species can be used successfully in arid soils phytoremediation and also in improving of the ambient air quality. Due to the chemical profile and to its behaviour concerning the noxious environmental factors, *P. atriplicifolia* can be widely used in urban areas characterized by serious pollution problems, contributing to the maintenance of biodiversity. It is also suitable for *xeriscaping* in order to lowering the water consumption, leaving more water available for other domestic and community uses and the environment.

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