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MORPHOLOGICAL-ANATOMICAL CHARACTERISTICS OF COMMON JUNIPER (*JUNIPERUS COMMUNIS*) FROM THE AREA OF MOUNTAIN KOPAONIK

Predrag S. VASIĆ[1], Marina D. TOPUZOVIĆ[2], Nenad Đ. LABUS[1] and Darko V. DUBAK[3]

[1] Institute of Biology, Faculty of Science, University of Prishtina, Lole Ribara 29, 38220 Kosovska Mitrovica, Serbia

[2] Institute of Biology and Ecology, Faculty of Science, University of Kragujevac, Radoja Domanovića 12, 34000 Kragujevac, Serbia

[3] Biotechnical Institute, Faculty of Science, University of Montenegro, Kralja Nikole b.b., 81 000 Podgorica, Montenegro

Key word:
Juniperus communis, morpho - anatomical characteristics, altitude.

SYNOPSIS

Morphological and anatomical characteristics of the leaves taken from the species *Juniperus communis* from mountain Kopaonik are analysed in this work.The level of distinctions between the leaves taken from various altitudes (420m-1420m) is determined by analysing three morphological and sixteen anatomical characters. The distribution of the centroids around the first and the second discriminant axis sharply distinguishes Common Juniper at this altitude from the samples taken from different altitudes.

Кључне речи:
Juniperus communis, морфо-анатомски карактери, надморска висина.

SINOPSIS

Морфолошко-анатомске карактеристике листа клеке (Juniperus communis) са подручја планине Копаоник

У овом раду анализиране су морфолошке и анатомске карактеристике листа врсте *Juniperus communis* са планине Копаоник. Ниво разлика између листова узетих са различитих надморских висина (од 420 до 1420 метара) утврђен је анализом три морфолошка и 16 анатомских карактера.

INTRODUCTION

The genus *Juniperus* L. belongs to the family of Cupressaceae. Within flora in Serbia, (J o v a n o v i c, 1992), this genus has nine species, six local and three park species (introduced). The genus *Juniperus communis* belongs to the group of phanerophyta (a bush or a short tree). The researches on this genus are mainly related to study of essential oils in leaves (A d a m s, 1998, A d a m s, 2000; M a t o v i c, M., 1997). For those reasons, our study of anatomic and morphological characters of Common Juniper leaf was extremely difficult, due to the lack of adequate literature data which would be used for comparing our results. Therefore, our research gets an additional significance, being a pioneer research, but at the same time it suffers additional problems, since it has to lay the foundations for studying morpho-anatomic characters of Common Juniper leaf in Serbia. A propos, we analyzed those characters from the aspect of their variability with the increase of altitude.

MATERIAL AND METHODS

Morphological and anatomic characters of genus *Juniperus communis* leaf were investigated on the material which had been collected in the period from 2001 to 2004. The plant material used for comparative anatomic analysis had been collected at the south-west part of the mountain Kopaonik and comes from different altitudes (420, 620, 820, 1020, 1220 and 1420 metres). (Figure 1). The collected material was fixed in the solution formalin-alcohol at the proportion 50%:50%. After that, it was conducted through series of alcohol of increasing concentration, due to dehydration. Permanent microscopic preparations were made on manual microtome. Sections of thickness 10-15μm were then coloured by safranin and light green.

Unused conserved plant material, as well as microscopic preparations, is located in Herbarium of the Department for Biology at the Faculty of Science in Prishtina, with head-office temporarily located in Kosovska Mitrovica. Measuring of dimensions of anatomic characters was performed with micrometer scale. For each anatomic character, 100 preparations were analyzed. In total, 16 anatomic and 3 morphological characters were analyzed. The measured anatomic characters are: leaf width (SL), leaf thickness (DL), front cuticle thickness (DKL), back side cuticle thickness (DKN), thickness of outer wall of front epidermis (DSZEL), thickness of inner wall of front epidermis (DUZEL), front epidermis width (SEL), back side epidermis width (SEN), front epidermis height (VEL), back side epidermis height (VEN), conductive vessel length (DPS), conductive vessel width (SPS), thickness of inner wall of back side epidermis (DUZEN), thickness of outer wall of back side epidermis (DSZEN), resin canal width (SSMK) and resin canal length (DSMK). Morphological characters are: leaf length (MDL), leaf width (MSL) and leaf thickness (MDBL).

Figure 1. Geographic position of locality where material had been collected.

Statistical analyses

The obtained values of morpho-anatomic characters were processed by programme package Statistics 6.0 for Windows (Statsoft 2001). Uni-variant descriptive statistical analysis was made for all samples, by which mean values and range were calculated. In order to determine variability as well as the degree of statistical differences between mean values of anatomic characters of Common Juniper leaf from various altitudes, variance analysis (ANOVA) and discriminative canonical analysis (DCA) were made. On the basis of calculated values of Mahalanobis distances, UPGA cluster analysis was done as well.

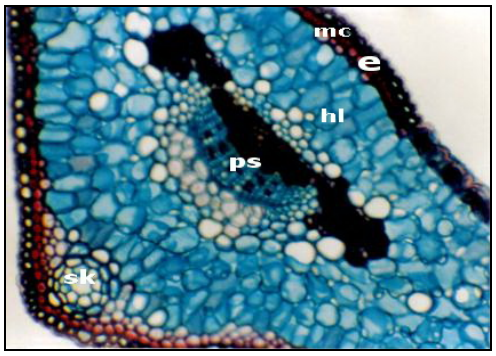
RESULTS

Morphological leaf properties

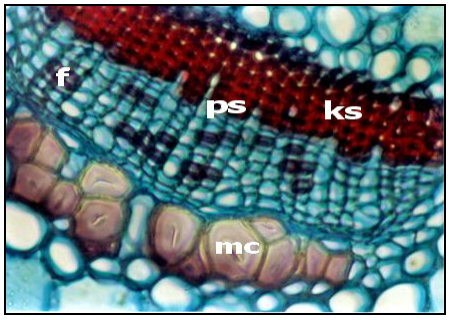
The leaf of Common Juniper (*Juniperus communis)* has the form of a needle; the needles are usually grouped as three whorl, bent, stiff and spiky needles. The length of analyzed leaves was, in average, from 11,1 mm to 15,1 mm, while the width of leaves was, in average, 1,0 mm to 1,4 mm. Leaves thickness was from 0,4 mm to 0,6 mm. It was established that the average leaf length continuously decreased with the altitude increase. On the other hand, width and thickness of leaves increased with the increase of altitude.

On the cross section of Common Juniper leaf three main layers were observed: front epidermis, mesophyll and back side epidermis (figure 2). Epidermis on front and

back side of the leaf is well developed and it forms the outer and inner wall. On its outer side, there is a well developed cuticle, whose thickness increases with the increase of altitude. In front epidermis, there are stomas which are a little bit retracted compared with epidermis cells. On the leaf back side, there is well developed resin canal. On leaf edges, collenchyma cells, distributed in several layers, can be observed. Mesophyll is well developed and made of spongy and palisade tissue. Spongy tissue is made of ball-shaped cells with intercellulars between them, while palisade tissue is made of rectangular-shaped cells. In the central part of the leaf, there is one conductive vessel of collateral type, with clearly differentiated xylem cells and phloem cells (figure 2B).



2A



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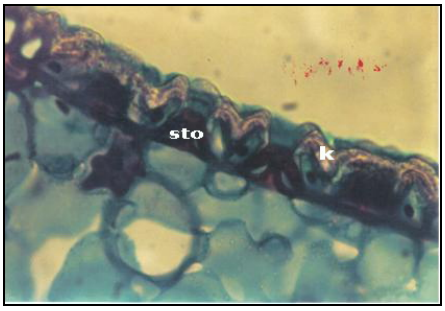

2C

Figure 2. Cross section of Common Juniper leaf: e – epidermis; mc – mechanical cells; hl – chlorenchyma: ps – conductive vessel; sk – resin canal; κs – xylem; f – phloem (increase x 20); k – cuticle; sto – stomas; (increase x 40).

Statistical analysis of morpho-anatomic characters

Descriptive statistics of anatomic characters has shown a continuous increase of their mean values with the increase of altitude. Anatomic characters have a rapid increase of values at 620 meters of altitude, where those values are the highest, as a rule. Starting with 820 metres of altitude up to 1420 meters of altitude, clear correlation of height increase and characters values can be observed (table 1, in enclosure). The smallest mean value of leaf width of 388,7 μm and leaf thickness of 131,7 μm was measured at the lowest point (420 meters of altitude). On the other

hand, the highest values of these two characters were observed at 620 metres of altitude (695,0 μm for leaf width, that is 264,1 μm for leaf thickness). Thickness of leaf front cuticle is the smallest at 420 metres of altitude (0,79 μm), and it is the largest at 1420 metres of altitude (1,26 μm). Thickness of back side cuticle is also the smallest at 420 metres of altitude (0,80 μm), while it is the highest at 1020 metres of altitude (1,57 μm). At 620 metres of altitude the following characters have the highest mean values: thickness of outer wall of front epidermis (0,52 μm), front epidermis width (7,38 μm), back side epidermis width (7,35 μm), front epidermis height (3,89 μm), back side epidermis height (3,44 μm), conductive vessel length (84,8 μm), conductive vessel width (40,7 μm), thickness of inner wall of back side epidermis (0,87 μm), thickness of outer wall of back side epidermis (2,84 μm), resin canal width (67,6 μm) and resin canal length(54,0 μm).

Variance analysis (ANOVA) has shown that there are statistically significant differences for all analysed leaf characters between samples taken from different altitudes (table 2).

Table 1. Descriptive statistics of anatomic characters of Common Juniper *Juniperus communis* leaf

Anatomic characters of the leaf (μm)		Altitude (m)					
		420	**620**	**820**	**1020**	**1220**	**1420**
SL	$\overline{X}$	388.7	695	401.6	505.9	490.8	481.9
	min	270	630	280	350	420	400
	max	430	780	540	670	630	660
DL	$\overline{X}$	131.75	264.1	154.05	193.7	189.1	187.9
	min	120	200	120	140	150	170
	max	160	350	200	270	220	210
DKL	$\overline{X}$	0.79	1.71	1.04	1.27	1.06	1.26
	min	0.41	0.83	0.41	0.41	0.83	0.83
	max	0.83	3.32	1.66	2.49	2.49	2.90
DKN	$\overline{X}$	0.80	1.45	1.32	1.57	0.97	1.18
	min	0.41	0.83	0.41	0.41	0.83	0.41
	max	1.66	3.32	2.075	2.49	1.245	2.49
DSZEL	$\overline{X}$	1.19	3.06	1.70	1.95	2.30	2.02
	min	0.83	1.24	0.83	0.83	1.24	0.83
	max	2.07	4.98	2.07	3.32	4.15	2.90
DUZEL	$\overline{X}$	0.41	0.52	0.415	0.45	0.70	0.60
	min	0.41	0.41	0.41	0.41	0.41	0.41

SEL	**max**	0.41	0.83	0.41	0.83	1.24	0.83
	$\overline{X}$	3.6	7.38	3.68	3.97	4.55	4.45
	min	2.49	4.15	2.49	2.49	2.07	2.49
SEN	**max**	4.98	9.13	4.98	5.81	7.47	6.22
	$\overline{X}$	3.57	7.35	3.51	4.46	4.41	4.73
	min	2.49	4.98	2.07	2.49	2.49	2.07
VEL	**max**	4.98	9.13	5.81	6.22	5.81	6.64
	$\overline{X}$	2.74	3.89	2.98	3.04	2.27	3.12
	min	1.66	2.49	2.07	1.66	0.83	1.66
VEN	**max**	4.15	5.81	4.15	4.56	4.15	4.57
	$\overline{X}$	2.45	3.44	2.56	3.05	2.66	2.94
	min	1.66	1.66	1.66	2.08	1.66	2.08
DPS	**max**	3.73	4.98	3.73	4.56	4.57	4.15
	$\overline{X}$	42.76	84.85	46.38	68.21	66.44	59.38
	min	34.86	55	29.88	36.52	49.8	48.14
SPS	**max**	51.46	110.0	74.70	106.24	86.32	76.36
	$\overline{X}$	20.70	40.70	24.40	29.23	27.36	27.94
	min	16.6	30	16.6	24.90	21.58	21.58
DUZEN	**max**	24.90	50.0	33.20	34.86	34.86	38.18
	$\overline{X}$	0.41	0.87	0.44	0.49	0.87	0.65
	min	0.41	0.41	0.41	0.41	0.41	0.41
DSZEN	**max**	0.41	2.07	0.83	0.83	1.66	1.24
	$\overline{X}$	1.35	2.84	1.76	1.93	1.44	2.1
	min	0.83	0.83	0.83	1.24	0.83	0.83
SSMK	**max**	2.49	4.15	2.49	3.32	3.32	2.49
	$\overline{X}$	39.6	67.60	40.10	45.30	53.48	53.05
	min	28.22	49.50	66.40	21.58	33.20	39.84
DSMK	**max**	53.12	63.91	69.92	74.70	74.70	88.00
	$\overline{X}$	40.63	54.04	44.35	48.80	56.14	53.02
	min	26.56	33.00	16.60	23.24	36.52	33.20
	max	54.78	53.98	63.80	74.40	84.66	74.70

Table 2. Values of Variance analysis tests for analyzed anatomic characters of Common Juniper leaves of samples from different altitudes.

Character	F test (df1,2)	p
SL	444.52	0.000 ***
DL	349.84	0.000 ***
DKL	49.72	0.000 ***
DKN	43.25	0.000 ***
DSZEL	134.76	0.000 ***
DUZEL	52.10	0.000 ***
SEL	308.25	0.000 ***
SEN	309.44	0.000 ***
VEL	36.03	0.000 ***
VEN	51.11	0.000 ***
DUZEN	49.89	0.000 ***
DSZEN	93.02	0.000 ***
DPS	46.02	0.000 ***
SPS	360.69	0.000 ***

Table 3 shows the results of discriminative analysis of anatomic characters of Common Juniper leaf in the space of the first three discriminative axes. In the table, it can be observed that the first axis describes 89,7% of the total discrimination. The width of conductive vessel (SPS) and width of front epidermis (SEL) mostly contribute to the discrimination of samples along this axis. The second canonical axis describes only 5,6% of the discrimination, and cumulatively with the first one 95,3%. All three axes in total describe 98,2 % of the discrimination. Front epidermis width (SEL) and back side epidermis width (SEN) along the third axis influence the discrimination predominantly.

Table 3. Loads of anatomic characters of Common Juniper leaf of samples from different altitudes.

Character	CV 1	CV 2	CV 3
SL	-0.222	-0.059	0.296
DL	-0.256	0.019	0.013
DKL	-0.116	0.139	-0.206
DKN	0.099	0.330	0.250
DSZEL	-0.221	-0.218	0.091
DUZEL	0.005	**-0.605**	0.251
SEL	**-0.526**	-0.149	**-0.510**
SEN	-0.321	0.121	**-0.470**
VEL	0.152	0.223	0.164
VEN	-0.015	-0.021	0.266
DUZEN	-0.277	-0.379	-0.100
DSZEN	-0.288	0.333	0.259
DPS	0.003	-0.178	0.139
SPS	**-0.421**	0.087	0.210
Eigenvalue	12.391	0.772	0.404
Cum.Prop.	89.7%	95.3%	98.2%

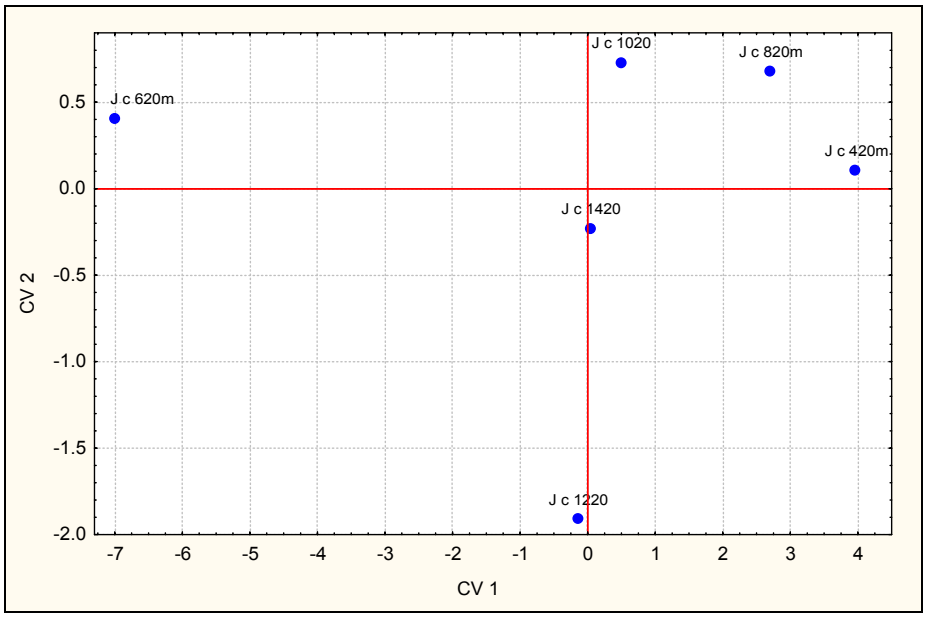

Figure 3. The position of centroids of analyzed Common Juniper samples from different altitudes in the space of the first and the second canonical axes.

From the graphic display of centroids position in the space of the first and second canonical axis, it can be observed that only the sample from 620 metres of altitude clearly differentiates along the first axis, and partly the one from 420 metres of altitude (figure 3). The samples from other altitudes are grouped. Only the sample from 1220 metres of altitude differentiates along the second axis. Along the third canonical axis (figure 4) the samples clearly differentiate into two groups: on one side there are samples from the smallest altitudes (420 and 620 metres), while on the other side there are samples from all other altitudes (820, 1020, 1220 and 1420 metres).

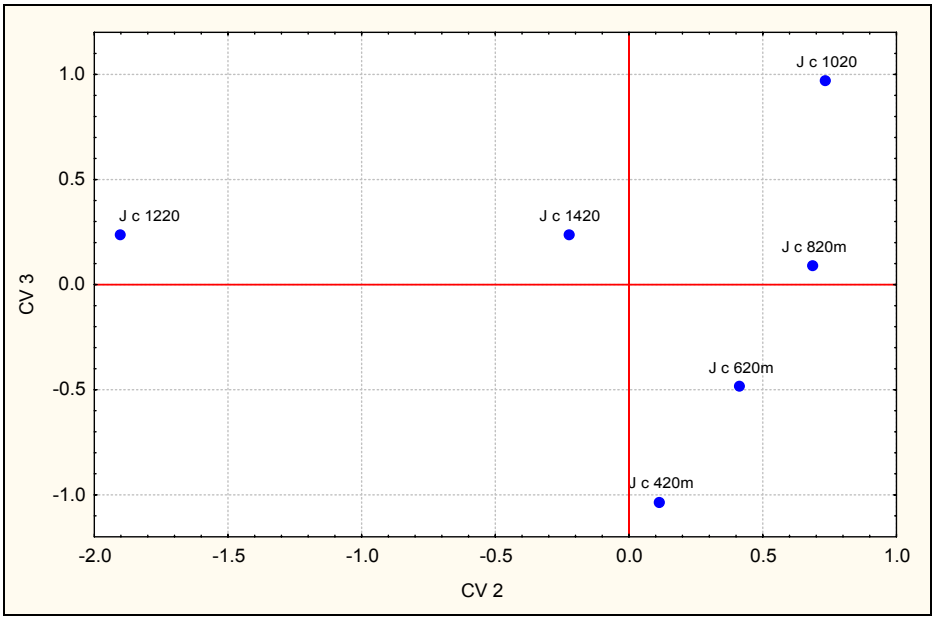

Figure 4. The position of centroids of analyzed Common Juniper samples from different altitudes in the space of the second and the third canonical axes.

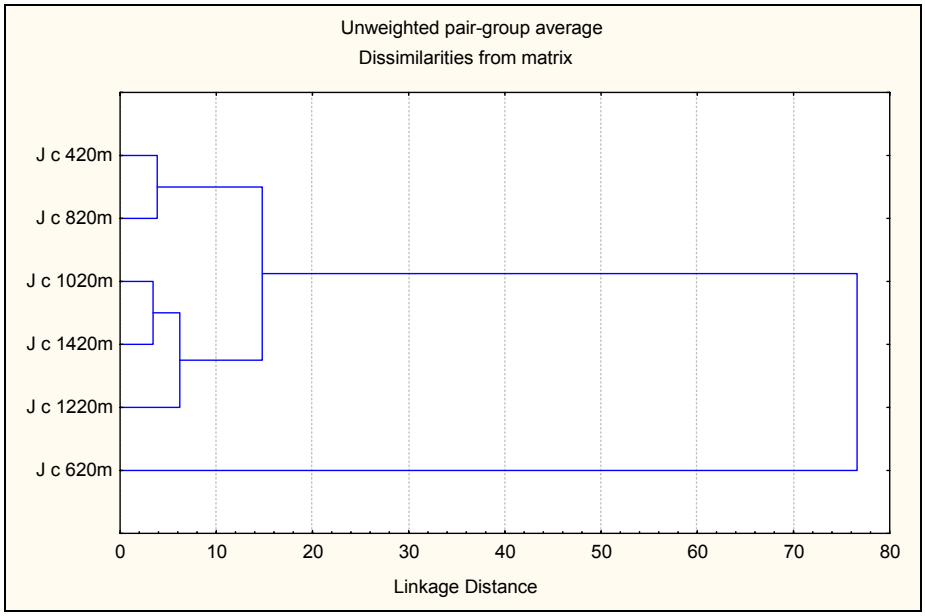

Figure 5. Cluster analysis of phenotype similarities of analyzed *Juniperus communis* samples from different altitudes.

Cluster analysis of samples of genus Common Juniper shown in figure 5, also demonstrated a clear phenotype differentiation of samples from 620 metres of altitude. The remaining samples were grouped into two subgroups: one consists of samples

from 420 and 820 metres of altitude, and the other one of samples from 1020, 1220 and 1420 metres of altitude.

DISCUSSION

With the aim of analyzing morphological and anatomic characters of Common Juniper leaf from the area of mountain Kopaonik, the samples taken from different altitudes (from 420 to 1420) were analyzed. By the application of uni-variant, bi-variant and multi-variant statistical methods, it was determined that the change of altitude influences the change of morphological and anatomic characters of Common Juniper leaf. The descriptive statistics showed that with the increase of altitude, leaf length decreases with simultaneous increase of thickness and width, which clearly indicates the intensification of xeromorphic properties with the increase of altitude.

The existence of this correlation can be explained by adapting to environment conditions imposed upon plants. Such active adapting makes the investigated species efficient and economical for exploiting the available resources of the environment and makes them competitive in relation to other habitat species.

These correlations in changes of morphological characters of the leaf with the increase of altitude, can be explained by the fact that in the mountain range, with the increase of altitude, going towards the top, the ecological conditions become less favourable due to which the trees change shape and size, becoming shorter, with smaller crown and smaller leaves (leaf length decreases and its width and thickness increase). Such changes contribute to better tolerance of low temperatures and water shortage.

The leaves collected at different altitudes clearly differ one from the other statistically for all anatomic characters. This was definitely confirmed by variance analysis (ANOVA).

By discriminative canonical analysis it was established that anatomic characters of epidermis are the major contributors to the discrimination along the first three axes. First of all, those are thickness of inner wall of front and back side epidermis, and width of front epidermis and width of back side epidermis also contribute to the discrimination significantly. In addition to epidermis characters, width of conductive vessel also contributes to the discrimination to a great extent.

The analysis of the graphic display of the centroid position in canonical axes space clearly shows the differentiation of sample from 620 metres of altitude. This is also in line with the results of the descriptive statistics which has also shown that the sample from this altitude clearly differs from the other samples. UPGA cluster analysis confirmed all this.

Therefore, it can be concluded that, on the basis of statistical processing (uni-variant, bi-variant and multi-variant statistic analysis) of anatomic characters of Common Juniper leaf of genus *Juniperus communis*, there is a clear differentiation of samples collected at different altitudes. This is due to the fact that the increase of altitude leads to changes of ecological conditions in the habitat, and plants, in order to survive in such conditions, have to adjust to those changes. That adjustability is

manifested by changes in morpho-anatomic characters of the leaf (increase of leaf width and thickness, increase of cuticle thickness, increase of epidermis wall thickness etc).

Bearing in mind that literature data on morpho-anatomic characters of Common Juniper leaf are scarce (R o t o n d i et al., 2003) , and in our country there was no data up to no now, we believe that the results obtained in this research will improve understanding of dependence of morphological and anatomic construction of Common Juniper and other conifers on change of ecological conditions in the habitat. We hope that they will serve as a good foundation for some future researches of anatomic and morphological characters of Common Juniper not only in the area of Kopaonik, but also on other localities where it exists.

LITERATURE

A d a m s, R. P. (2000): Systematics of *Juniperus* section *Juniperus* based of leaf essential oils and random amplified polymorphic DNAs (RAPDs). Biochemical Systematics and Ecology 28, 515-528.

A d a m s, R.P. (1999): The leaf essential oils anad chemotaxonomy of *Juniperus sect.* Biochemical-and-Ecology. 1998,26:6, 637-645; 17 ref, USA

C a s t r o D. P., V I l l a r S a l v a d o r, P., P e r e z –R o n t o m e, C., M a r t i n e z, M., M o n t s e r r a t M. G. (1997): Leaf morphology and leaf chemical composition in three *Quercus* (Fagacee) species, along a rainfall gradient in NE Spain. Trees 11, 127-134.

J o v a n o v I c, B. (1992): Genus *Juniperus* L. In Saric, M (ed): Flora of Serbia 1 214-224. SANU. Belgrade

K o r n, W. R. (2002): Chimeric patterns in *Juniperus slinensis* Torulosa Variegeta (*Cupressaceae*) expressed during leaf and stem formation. Department of Biology, Bellamine University, Louisville, Kentusky 40205 USA.

K o r n. R. M. S p a l d i n g (1973): The geometry of plant epidermal call. New Phytologist 72, 1357-1365.

R o t o n d i, A., R o s s i, F., A s u n i s, C., C e s a r a c c i o, C. (2003): Leaf xeromorphic adaptations of some plants of a coastal Mediterranean macchia Ecosystem. Journal of Mediterranean Ecology, Vol. 4, N_o 3-4, 25-35.

S a t i l, F., S e l v i, S. (2007): An anatomical and ecological study of some *Crocus* L.taxa (Iridaceae) from the west part of Turkey. Acta Bot. Croat. 66, (1), 25–33.

Матовић, М. (1997): Infusible technology in processing Common Juniper products (*Juniperus communis* L.). Kragujevac.