HODNOTENIE CUPRESSUS SEMPREVIRENS L. OTUŽILOSŤ CEZ SACHARIDOV A OBSAHU PIGMENTOV V LISTOCH

ASSESSMENT OF CUPRESSUS SEMPERVIRENS L. HARDINESS THROUGH CARBOHYDRATES AND PIGMENTS CONTENT IN THE LEAVES

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The study was carried out in 2011 – 2014 year at Botanical garden of Slovak University of Agriculture in Nitra, Slovak Republic. *Cupressus sempervirens* L. was planted in two types of planting, plants planted direct in the soil and stayed outside during winter time and plants planted in pots and protected during winter time, when plants were removed in to greenhouse from end of November until end of March. The sample of young, one year leaves had been taken in end of January when temperature over night was (-7°C) and at 9 am was (-3°C). The results showed that there are significant differences between plants planted outside and plants protected during winter time in all of studied characteristics. Plants planted in ground had the highest chlorophyll a and total sugar content in comparison with plants in pots which were in greenhouse when temperature has recorded on (8°C) in average. There is an inverse relationship between chlorophyll a and (total sugar and starch). The study found also an inverse relationship between total sugar and starch content in the leaves. Also an inverse relationship between chlorophyll a and chlorophyll b was found; increase of chlorophyll a leads to decrease of chlorophyll b content.

Keywords: *Cupressus sempervirens* L. leaves, plant pigments, sugar, starch

Cupressus sempervirens var. pyramidalis L. native to Mediterranean, the Italian Cypress is surely one of the most beautiful and evocative trees in the world it grows in different parts of many countries (Blakelock, 1979). Cupressus is a tall and erect coniferous evergreen tree with dark green foliage all seasons and spherical green cones maturing brown. It may reach a height of 20 meters. It is very common in boulevards, ornamental gardens, planted forests, cemeteries and as a windbreaker around plantations. It does best in rich, fertile soils and sun or partial shade places. Within photosynthesis process the plants take in CO2 and water (H2O), and utilizing the power of sun light along with minerals, enzymes and chlorophyll, produce oxygen (O2) and carbon compounds such as glucose (C6H12O6). Plants can produce a variety of carbon compounds through this process, including oils, proteins, and starches. Plants use these compounds to build all of their materials for survival and reproduction. We use these materials for our food, medicines, dyes, perfumes, fibers etc. Both biotic and a biotic stress factors affect the content and efficiency of leaf photosynthetic pigments or their reciprocal ratio (Bacci et al., 1998). For example,

the assessment of leaf photosynthetic pigments is an important indicator of senescence because breakdown of leaf chlorophyll is associated with environmental stress (Brown et al., 1991).

(Rose & Haase, 2002) reported that chlorophyll fluorescence measurements are useful for determining cold hardiness and resistance to stress, because they provide a rapid assessment of seedling vigour following exposure to freezing. Sun leaves are known to differ from shade leaves in their composition of photosynthetic pigments, chloroplast ultra structure, photosynthetic rates, and resistance to light stress (Sarijeva et al. 2007).

Carbohydrate contents of forage can vary widely due to the interaction of plants and their environment. These variables include: species and variety of the forage, stage of growth, and environmental conditions during plant growth. Environmental factors include temperature, light intensity and availability of water and nutrients. Temperature has been shown to have an effect on mobilizing carbohydrate from leaves (Hartt, 1965; Potvin & Strain, 1985). There have been critical evaluations and strong overtones throughout on the role of the principal climatic that influence growth through the intermediation of internal bio physicochemical processes such as photosynthesis and carbon balance (Körner, 2003). For example, temperature influences rates of photosynthesis and respiration, as well as growth rate associated with rates of cell division and elongation. Levels of soluble sugars and starch reflect the balance between carbon gain (photosynthesis) and loss (growth and maintenance respiration) within a plant, representing a tree's capital for growth after dormancy and acting as a buffer for insufficient source activity (photosynthesis) due to adverse weather conditions or loss of foliage. In the green house, day/night temperatures are maintained at specific levels for each plant to obtain profitable yield and marketable quality at prevailing solar radiant energy levels.

The aim of this study is to determine the total soluble carbohydrates (starch and total sugar) and pigment contents of Cupressus sempervirens which was planted under Slovak Republic climate condition.

MATERIAL AND METHOD

The study was carried out in 2011 – 2014 year at Botanical garden of Slovak University of Agriculture in Nitra, Slovak Republic. *Cupressus sempervirens* var. pyramidalis was planted in two types of planting and in three replications for each planting type. First plants planted in the ground and stayed outside during winter condition, the second was planted in pots and protected during winter time, and the plants remove in to greenhouse from end of November until end of March. The sample of leaves was taken when the temperature was (-3°C) at 9 am and during night was (-7°C) outside, the young leaves was taken from plants and the pigments, total sugar and starch was determined.

Assimilation pigments contents were measured in control leaves as follows: The segments of the youngest mature leaves of Cupressus sempervirens L. were homogenized with using sea sand, MgCO3 and 100% acetone and then extracted with 80% acetone. Extracts were centrifuged 2 minutes at 2500 rpm. Absorbance (A) of the solution was measured by UV-VIS spectrophotometer (Jenway, UK), at 470 nm, 647 nm, and 663 nm, with correction for scattering at 750 nm; the measurements were done in three repetitions (OLŠOVSKÁ et al. 2013). The concentrations of chlorophyll *a* (Chl *a*), chlorophyll *b* (Chl *b*) and carotenoids (Car) in mg*l-1 was determined by using the equations of Lichtenthaler (1987):

Chl *a* = 12.25*(A_{663} - A_{750})-2.79*(A_{647} - A_{750})*D

Chl *b* = 21.50*(A_{647} - A_{750})-5.10*(A_{663} - A_{750})*D

Chl *a*+*b* = 7.15*(A_{663} - A_{750}) +18.71*(A_{647} - A_{750})*D

Car = [(1,000*(A_{470} - A_{750}) -1.82*(Chl *a*) - 85.02*(Chl *b*))/198]*D

The concentrations of the pigments were calculated in mg dm-3; An was the absorbance at given wavelengths (n) after correction for scattering at 750 nm; D was the optical thickness of cuvette; results were also recalculated in mg. m-2 using the volume of solution and the area of leaf segments: [mg. m-2] = V/1000*1/A, when V is volume of 80% acetone and A is area of leaf segments.

The starch content was determined according to the polarimetric method of Ewers (Michalik et al., 1978). A portion of 5 g of a homogenised sample is weighed in a 100 ml Kohlrausch volumetric flask and its content is mixed with 25 ml of 1.124% HCl solution. After addition of another 25 ml of 1.124% HCl solution, the suspension is heated on a boiling water bath for 15 min (after 3 min the content of a volumetric flask is mixed to avoid coagulation). Once the hydrolysis is finished, 20 ml of 1.124% HCl solution is added. After fast cooling (using a stream of flowing water), clarification using 5 mL of Carrez I (30% ZnSO4 solution) and 5 ml of Carrez II (15% K4[Fe(CN)6] solution) solutions is performed. Finally, a volumetric flask is filled up by distilled water, its content is properly mixed, and filtrated using a filtration funnel. The obtained filtrate is then transferred to a polarisation tube (2 dm) and measured using a polarimeter.
The extent of polarisation is related to the concentration of the optically active molecules in solution by the **Eq. 1**

$$\alpha = [\alpha]^t_\lambda . \ell . c$$

Where α is the measured angle of rotation, $[\alpha]^t_\lambda$ is the optical activity (which is a constant for each type of molecule), ℓ is the path length and c is the concentration. The overal angle of rotation depend on the temperature and awvelength of light used and olso these parameters are usually standarddised (e.g. 20°C and 589.3 nm (the D-line for sodium).

The obtained value is firstly corrected for a laboratory temperature (t) drift using **Eq.2**

$$\alpha \text{ crrected} = \alpha \text{ meaured } (^{\circ}S) - 0.0144(t-20)\ [^{\circ}S]$$

followed by multiplying by a factor of 0.3462.

The amount of starch (X) in the sample is calculated using **Eq.3**

$$X = \frac{10^4.\alpha}{[\alpha]^t_\lambda.\ell.m}$$

Where α is calculated value of optical rotation, $[\alpha]^t_\lambda$ is the optical activity (specific rotation) depending on the discharge lamp and wavelength of light used and variety of starch, ℓ is the path lenght (2dm), and m is the sample weight (5g). For a mercury discharge lamp and a wavelength (λ) of 546.1 nm, the $[\alpha]^t_\lambda$ values are 214.7, 216.3, 213.3, 213.1, 218.5,217.0 and 215.5 for wheat, rye, barly, oat, rice, maize and unknown cereal starch, respectivly. (Not: the correction for moisture is not account in the equation).

Total sugar was determined according to Somogyi (in MichAlik et al., 1978 and Frederick, 1989) . 1-ml samples were combined either with 10 mg substrate and 1 ml citrate buffer (0.1 M, pH 5.0) or with 1 ml substrate solubilized in 0.1 M citrate buffer (pH 5.0) at 1% (w/v) in 50-ml Folin tubes. A control for each sample was prepared with substrate and buffer. Tubes were incubated at 40°C for 24 h. After incubation, 2 ml of copper reagent, consisting of 4 parts KNa tartarate: Na2C03:Na2SO4:NaHCO3 (1:2:12:1.3) and 1 part Cu- SO4 · 5H2O:Na2SO4 (1:9), was added to each tube. Both copper reagents must be prepared by boiling to completely dissolve the components; they can then be stored at room temperature. They were mixed together just prior to use. After 1 ml of sample was added to the appropriate control tubes, all tubes were boiled for 10 min in a water bath. The tubes were then cooled completely, 2ml of arsenomolybdate reagent (25 g ammonium molybdate in 450 ml H2O + 21 ml H2SO4 + 3 g Na2HASO4 · 7H2O dissolved in 25 ml H2O) was added to each tube, and the tubes were shaken thoroughly before adjusting the final volume to 25 ml with water. Individual samples were filtered through filter paper, and colorimetric measurements were determined by transmitted light at 500 nm in a spectrophotometer. The results of sugar and starch content in leaves are expressed in% of dry weight (d w). Evaluated the nutrient system of soil samples taken from the experimental area. Sampling took on 11.12.2012, from the direct planted plants and plant planted in pots was collected from a depth of 50 mm and 300 mm. (Table 1, 2) Nutrients and trace elements in soil samples In the evaluation of elements of the samples taken from the experimental area was determined acceptable nutrient content according to Mehlich III. The values of the elements of soil analysis for the experimental area were compared within the assessment criteria analysis results of soil Mehlich III method. and according to Lindsay and Norvel under Annex no. 5 to

Decree No. 338/2005 Coll. Ministry of Agriculture of the Slovak Republic on 6 July 2005 on the procedure for the collection of soil samples. Air temperature and rainfall were received from dates of metrological station of Botanical garden during study period (table 3, 4).

An experiment was laid out as Factorial Randomized Complete Design (RCD) in three replications, the data were analyzed with the general linear model procedures in Statistical Analysis System (SAS), and Duncan test at level 0.05 was used for the means separation.

RESULTS AND DISCUSSION

Data obtained that planting outside increased all pigments and total sugar while planting in pots and protected during winter time increased only starch content in plants. Chlorophyll content is of particular significance to precision in agriculture as an indicator of photosynthetic activity. Nitrogen concentration in plants is related to Chlorophyll content and indirectly to one of the basic plant physiological processes photosynthesis (Sabo et al., 2002; Bojovic & Stojanovic, 2005). As it seen in table (2) that nitrogen level in pot plants was in good level in each depth (0-50mm, 0-300mm), while in plants planted in ground the soil in depth (0-50mm) nitrogen was suitable but in depth (0-300mm) nitrogen level was low, as it known that in agricultural and horticultural systems, mineral-N is mainly prone to losses through: (a) ammonia volatilisation; (b) leaching (i.e. removal in drainage water) and (c) denitrification (i.e. transformations into gaseous forms) (Cameron et al. 2013).

(Fig.1) described the amount of chlorophyll *a*, the maximum content of chlorophyll a was in plants planted outside during winter time (1.959mg.g-1) and the minimun chlorophyll a was in plant planted in pots and protected against winter condition(0.444mg.g1). The maximum total sugar was in plants planted in the ground and was outside in winter time (6.13 % dry weight) while lowest total sugar was in pot plants (2.81 % in dry weight) Some Chlorophyll a fluorescence ratios are frequently used to evaluate stress conditions (De Oliveira et al. 2009), This means that the hardiness in cupressus was icreased with increasing of Chlorophyll *a* . The ability of woody perennials to survive winter is depending on their entry into dormancy state as well as the development of their cold acclimation achieved by a continuous exposure from -5 to -15°C. In late autumn, after leaves have dropped and with the first frosts (up to -3°C), trees become dormant (WEISER, 1970). The results agree with (Pressman et al., 1994). Long term low temperature treatment led to a sharp decrease in the sugar content in Asparagus. Cold acclimation is associated with changes at morphological, metabolic, proteomic and gene expression levels. Among other changes, cold-acclimated plants can cope with the cold-impaired photosynthetic components such as induced changes in pigment complexes, reduced net and maximal photosynthetic rates, induced losses of photochemical efficiency, restricted electron transport and enzyme activity or reduced carbohydrate

metabolism. The data in (Fig.2) showed the effect of chlorophyll a on starch content in plants reduces in chlorophyll a laid to increase starch content in each planting types. Our data, along with those of Lefebvre et al., (2005), demonstrate a clear correlation between increased photosynthesis and starch accumulation.

An inverse relationship between chlorophyll a and chlorophyll b was found in (Fig.3). Maximum content of chlorophyll a was (1.959 mg.g-1) which were planted outside and the lowest chlorophyll b was in plants planted in pot (0.173 mg.g-1), (Table 5, 6). The results agree with Kalaivani et al. (2013). Leaves exhibit a structural and functional acclimation of the photosynthetic apparatus to the light intensity experienced during their growth (PRIOUL et al. 1980). Chlorophyll b is synthesized from chlorophyll a by oxidation of a methyl group on the B ring, of the latter molecule, to a formyl group at that position (Porra et al. 1993). The main function of chlorophyll b is to gather light energy and transfer it to chlorophyll a (Biswal et al. 2012). Chlorophyll b is important for plant in addition Sakuraba et al. (2012) have demonstrated that increased chlorophyll b synthesis delays senescence; thus, it retains the gene expression of several chlorophyll biosynthetic enzymes. These findings demonstrate the existence of a regulatory network among genes coding for enzymes involved in the greening process.

Chlorophyll *a+b* increased with decreasing chlorophyll a and chlorophyll b, the highest chlorophyll *a+b* was in plants planted outside during winter time (Fig. 4, 5).

The study of Shlyk et al., (1963) suggest that chlorophyll b is formed from newly synthesized molecules of chlorophyll a, rather than from the general pool of chlorophyll a molecules which are found by photo conversion and phytzlation of protochlorophyllide already existing in etiolated plants.

An inverse relationship was among carotenoids, starch and total sugar (Fig. 6) decreasing in starch (3.46 % in dry weight) laid to increase total sugar (6.13% in dry weight) and decrease carotenoids (o.252 mg. g-1) (Table.5, 6). In plants planted in pots and protected against cold weather, while decreasing in total sugar (2.81% in dry weight) increased starch content (6.37% in dry weight) and decreased carotenoids (0.782 mg. g-1). The concentrations of leaf pigments can be associated with environmental factors such as ambient temperature/sunlight (Saenger, 2002), water availability (Lacerda, 2002). As it known temperature is especially important, the rate of chemical reactions increases with warmer temperatures, and nearly all physiological processes involved in plant growth are controlled by temperature. As it showed at Fig. 7 the relation between chlorophyll a, chlorophyll b and carotenoids the chlorophyll a rate was highest than chlorophyll *b* and carotenoids the result agreeing with He et al. (1999) The content of chlorophyll *a* in green plants is twice as that as of chlorophyll b. The transfer of excitation energy from carotenoids to chlorophyll *a* is facilitated by the presence of chlorophyll *b* (Thorne & Boardman, 1971). Carotenoids accumulation differed with time (Watada et al. 1976).

Table 1 Nutrients and trace elements in soil samples (Department of Agrochemistry and Plant Nutrient of FAaFR, SAU Nitra, 2013 according to Mehlich III

Planting type	Depth mm	pH	Nan mg.kg-^{1}	The nutrient content mg.kg^{-1} **(Mechl.III)**			
				P	K	Ca	Mg
Planting in the ground	0-50	6,86	13,5	162,5	700	4710	952
Planting in the ground	0-300	6,88	8,6	111,25	537,5	4665	981.5
Planting in pots	0-50	5,87	27,6	465	900	6700	1369
Planting in pots	0-300	6,41	25,35	350	712,5	6635	1219

Table 2 Evaluation of analyses of soils for arable land (nutrient content limits in mg.kg-1) according to Mehlich III

Planting type	Depth mm	pH	Nan mg.kg-^{1}	The nutrient content mg.kg^{-1} **(Mechl.III)**			
				P	K	Ca	Mg
Planting in the ground	0-50	Neutral	Suitable	Very high	Very high	Very high	Very high
Planting in the ground	0-300	Neutral	Low	Very high	Very high	Very high	Very high
Planting in pots	0-50	Slightly acid	Good	Very high	Very high	Very high	Very high
Planting in pots	0-300	Neutral	Good	Very high	Very high	Very high	Very high

Table 3 Average temperature in °C Nitra (2011, 2012, 2013 and 2014)

Month	2011	2012	2013	2014
January	-0.90	1.36	-0,8	2,42
February	-0.60	-2.49	1,5	3,86
March	5.90	7.41	3,1	-
April	12.70	11.23	12,1	-
May	15.80	17.29	15,6	-
June	19.80	20.86	19,3	-
July	19.70	22.77	22,8	-
August	20.90	21.47	21,9	-
September	17.70	17.02	14,7	-
October	9.90	10.46	12,1	-
November	3.00	7.45	6,8	-
December	2.20	-0.91	2,3	-
Year Average Temperature	10.51	11.16	11,0	-

Table 4 Sum of rainfalls in mm Nitra (2011-2012- 2013 and 2014)

Month	2011	2012	2013	2014
January	25	61.1	71.2	1,19
February	6	23.5	75.6	1,1
March	27	2.8	113.9	-
April	13	36.1	20.4	-
May	48	19.6	77.8	-
June	91	70.1	46.7	-
July	122	61.4	2.1	-

August	152	7.3	73.9	-
September	92	32.7	60.0	-
October	37	76.1	30.5	-
November	1	34.6	71.3	-
December	42	44.4	11.0	-
Year Sum of Rainfalls	656	469.7	654.4	-

Table 5 Dry weight , starch and total sugar in leaves of *Cupressus sempervirens* L influenced by planting type

Planting type	Studied characteristics		
	Dry weight (mg)	Starch (% in dry weight)	Total sugar (% in dry weight)
Planting in the ground	92.063[b]	3.46[b]	6.13[a]
Planting in pots	92.393[a]	6.37[a]	2.26[b]

*Means not followed by the same letters are significant at 5% level of probability

Table 6 Chlorophyll *a,b, a+b* and carotenoids in leaves of *Cupressus sempervirens* L. influenced by planting type

Planting type	Studied characteristics			
	Chlorophyll *a* (mg. g^{-1})	Chlorophyll *b* (mg. g^{-1})	Chlorophyll *a+b* (mg. g^{-1})	Carotenoids (mg. g^{-1})
Planting in the ground	1.959[a]	0.863[a]	2.822[a]	0.782[a]
Planting in pots	0.444[b]	0.173[b]	0.617[b]	0.252[b]

*Means not followed by the same letters are significant at 5% level of probability

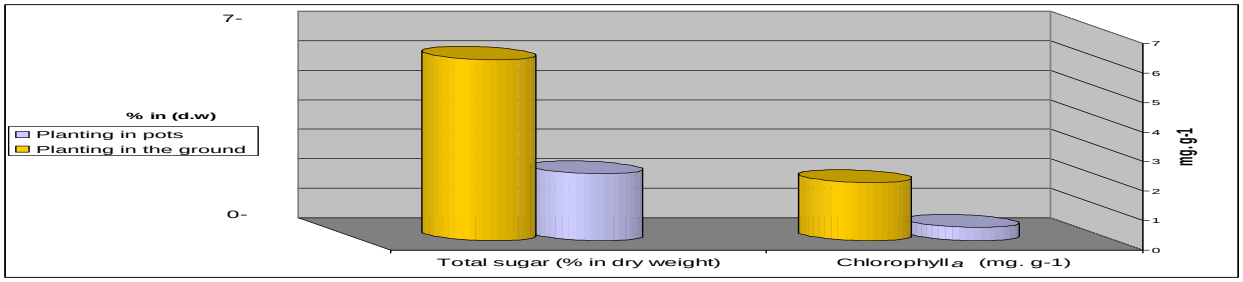

Figure 1 Chlorophyll *a* and total sugar content in leaves of *Cupressus sempervirens* L. influenced by planting type

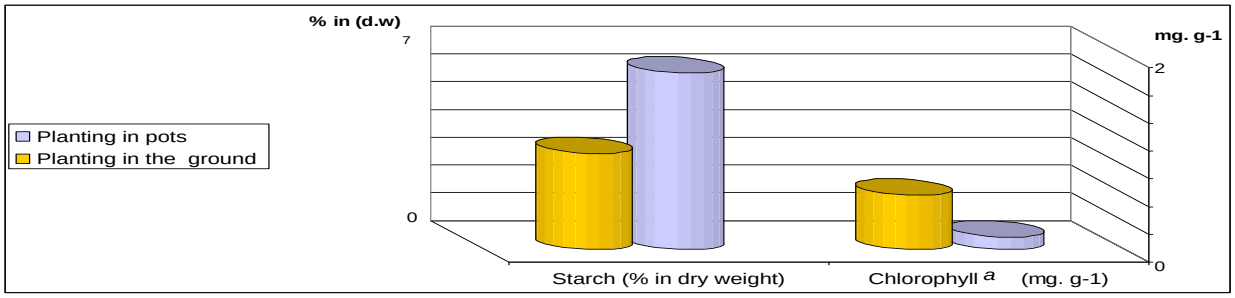

Figure 2 Chlorophyll *a* and starch content in leaves of *Cupressus sempervirens* L. influenced by planting type

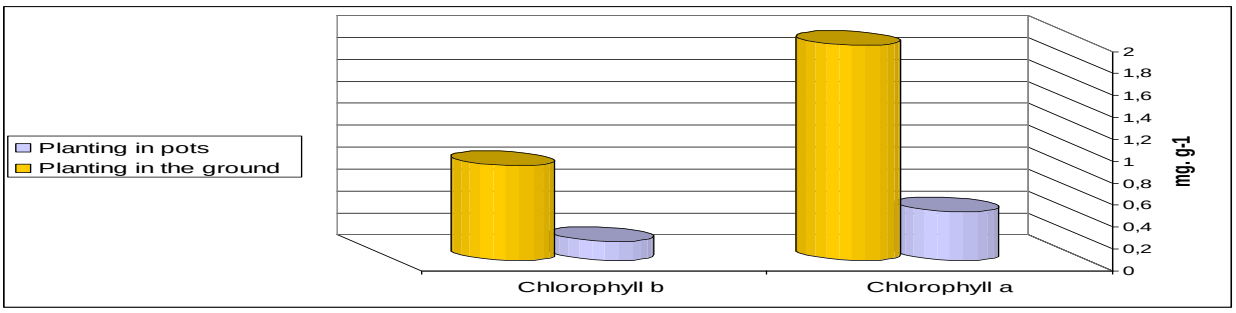

Figure 3 Chlorophyll *a* and chlorophyll *b* content in leaves of *Cupressus sempervirens* L. influenced by planting type

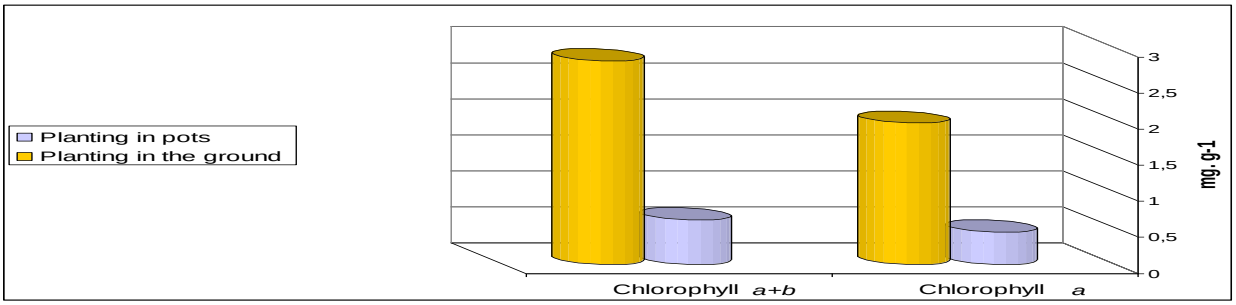

Figure 4 Chlorophyll *a* and chlorophyll *a+b* content in leaves of *Cupressus sempervirens* L. influenced by planting type

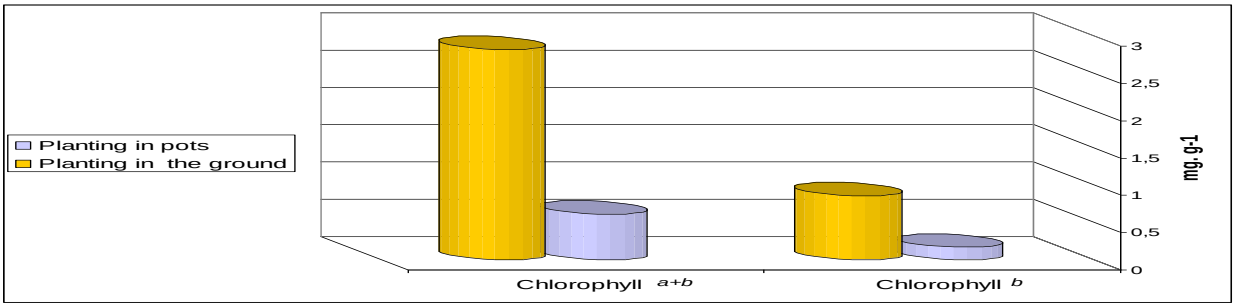

Figure 5 Chlorophyll *b* and chlorophyll *a+b* content in leaves of *Cupressus sempervirens* L. influenced by planting type

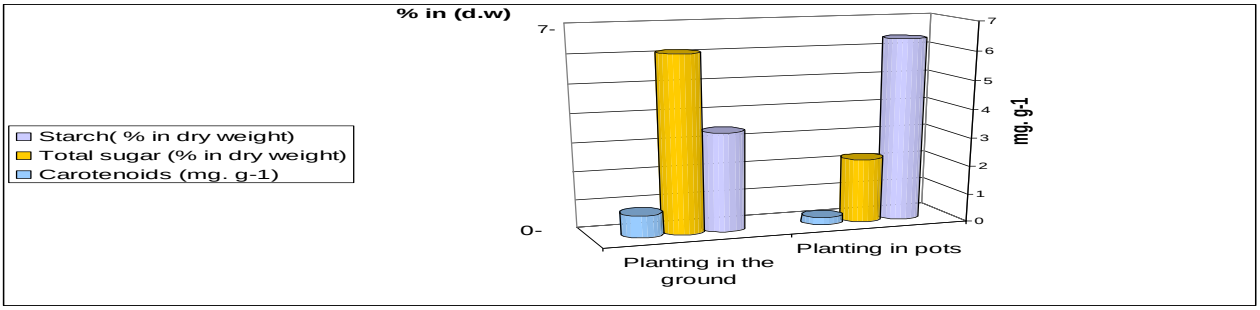

Figure 6 Starch, total sugar and carotenoids content in leaves of *Cupressus sempervirens* L. influenced by planting type

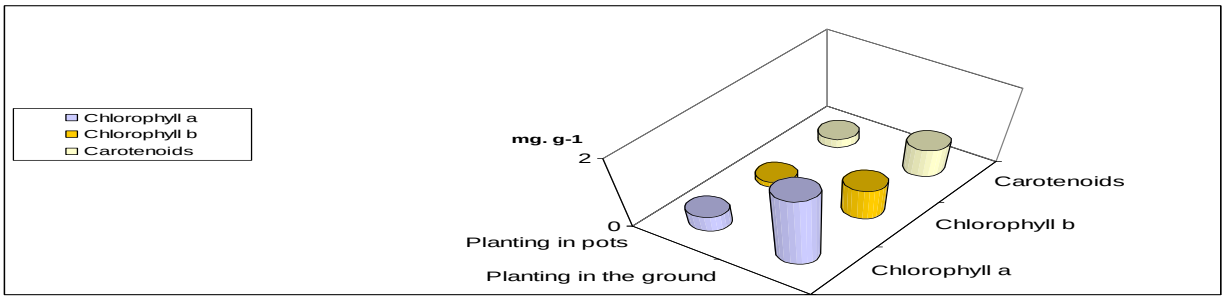

Figure 7 Chlorophyll *a*, chlorophyll *b* and carotenoids content in leaves of *Cupressus sempervirens* L. influenced by planting type

CONCLUSION

Štúdia bola vykonaná v roku (2011 - 2013) v botanickej záhrade Slovenskej poľnohospodárskej univerzity v Nitre , Slovenská republika . *Cupressus sempervirens* L. bol zasadený do dvoch typov pestovania , rastliny vysadené priamo v pôde, ktoré zostali vonku aj v zimnom období a rastliny vysadené v kvetináčoch a chránených v zimnom období a prenesené do skleníka od konca novembra do konca marca, kde teplota vzduchu dosahovala hodnoty +5 až +8 °C. Vzorky listov boli odobraté v druhej polovici januára, keď vonkajšia teplota vzduchu bola pod nulou (-7 °C v noci a -3 °C o 9:00hod. ráno). Výsledky ukázali , že existujú významné rozdiely medzi rastlinami vysadených na pokusnej ploche a rastlín chránených v zimnom období vo všetkých sledovaných charakteristikách. Dreviny vysadené na experimentálnej ploche mali vyšší obsah chlorofylu *a* celkových cukrov

v listoch v porovnaní s rastlinami vo vnútri. Existuje inverzný vzťah medzi obsahom chlorofylu *a* obsahom celkových cukrov a škrobu. Výsledky výskumu ukázali tiež inverzný vzťah medzi obsahom celkových cukrov a obsahom škrobu v listoch drevín. Rovnako bol identifikovaný inverzný vzťah medzi obsahom chlorofylu *a* a chlorofylu *b,* nárast chlorofylu a vyvolal zníženie obsahu chlorofylu*b*.

Kľúčové slová: *Cupressus sempervirens* L.,listy, rastlinné pigmenty, celkový cukor, škrob

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