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FRESH YIELD BIOMASS IMMORTELLE AND ESSENTIAL OILS CONTENTS DEPENDING ON THE GROWING LOCALITY

Abstract

Experimental trips with immortelle they were carried out at two sites: Kacarevo (44° 57'59 "N 20° 41'22" E, municipality of Pancevo, South Banat, Serbia) and Lešje (43°51'10"N 20°41'22" E, Municipality of Paracin, Pomoravlje District, Šumadija and Western Serbia), on two types soil (chernozem and grazing). Needs for quality Helichrysum essential oil in the world growing, because they are more and more use in many branches industrial processing. Total yield of fresh biomass and the content of essential oil immortelle he was in borders of maximum production, which in these plant species starts from third year and it takes about 10 years. Italian immortelle can be successful cultivated in continental areas, in this case in the mountain area Central Serbia and flatland Vojvodina.

Key words: Italian immortelle, agroecological conditions, yield of fresh biomass, content of essential oil, Serbia

INTRODUCTION

Immortelle - *Helichrysum arenarium* (L.) Moench is aromatic perennial shrub plant, light green leaves and golden yellow floral heads. It belongs to the genus Helichrysum and *Asteraceae* family. In the Mediterranean region, the genus is represented by about 25 autochthonous species. Mediterranean immortelle is a xerophytic species, which naturally grows on dry, sandy and stony areas Mediterranean region. That trait allows to growing in a wide range above sea level, between the sea level and 2200 m. It's widespread in countries of southern Europe, Northwestern Africa and Small Asia. Is used and for medical purposes, regenerative acting on damaged skin. Metabolites isolated from immortelle have a strong biological activity such as antimicrobial, antivirus, antivirus and antioxidant activity. Distillation of fresh plant material an essential oil is obtained, which it shows favorable medical-medical profile in therapeutic application (Miloradović et al., 2018).

The inflorescence of the *Helichrysum arenarium* (L.) M. is traditionally used in liver and biliary tract diseases, showing choleretic, cholagogic, and spasmolytic activity as well as stimulating digestion (Ożarowski, 1980). This plant raw material is known for its anti-inflammatory, antioxidant, and detoxifying properties. The main active compounds are flavonoids, but the above-mentioned species also contains other phenolics, such as α-pyrone derivatives, phthalides, coumarins, and phenolic acids (Czinner et al. 2002; Lemberkovics et al. 2002; Sroka et al. 2004; Bryksa-Godzisz et al. 2006). This heliophilous species has rather high thermal requirements, prefers warm regions and microhabitats as well as dry oligotrophic soils poor in organic matter (Pacholak, Załęcki, 1979). According to the Polish Pharmacopoeia (Geszprych et al. 2003), the inflorescence of yellow everlasting should contain in dry matter at least 0.5% of flavonoids expressed as quercetin equivalent. More detailed studies of 22 populations (Bryksa-Godzisz et al. 2006) showed a relatively large variation range of the amount of total flavonoids (0.15–0.78%) and phenolic acids (0.45–1.52%) (Forycka et al., 2016).

The aim of this paper was to determine the impact of locality on productivity of biomass and immortelle essential oil.

MATERIALS AND METHODS

Experimental trips with immortelle (*Helichrysum italicum* (Roth) G. Don) they were carried out at two sites: Kačarevo (Pančevo municipality, 44 ° 57'59 "N 20 ° 41'22" E, Vojvodina, District - South Banat, Serbia) and Lešje (43 ° 51'10 "N 20 ° 41'22" E, Paraćin municipality, Šumadija and Western Serbia, Pomoravlje District, Serbia), on two types of land (Chernozem and brown forest soil). On both test fields before the crop the winter wheat. During the autumn, classical basic cultivation of soil. In both experimental fields before the crop the winter wheat. During the autumn classical basic cultivation practice of soil with simultaneous introducing 300 kg ha^{-1} mineral

nutrient NPK 15:15:15 mixed with Protect Forte. This preparation was used to make equalize the carbonate pH of the soil growth on both types of soil. Immortelle production was realized in two phases. The first phase was carried out production of seedlings in the experimental greenhouse P. G. Dalibor Suša in settlement Altina (municipality of Zemun) in fully controlled conditions of heat, humidity and plant protection from pests and pathogens. The seed was sown at the end of December 2015, the transplant – pricking in containers in mid-February 2016. At the beginning of the second week of April nursery is presented and to adapt to the conditions of the outside environment. In the second phase, plants were planted 2016 at two sites. During the spring, ie before planting, the soil is prepared for planting plants. The growth was done manually at an intermediate distance of 70 cm and the distance between the plants in the order of 40 cm in order to achieve the planned density of crops of 35,000 plants per hectare. About 30 days before planting containers are brought to the open space for the adaptation of plants on the conditions of the external environment. Manual disassembly was done in two hands, due to different weather conditions. The paper is analyzed productivity parameters of immortelle in the third year of production (2017), plants are from seedlings planted 2015. Planting was carried out at the end of April 2016, manually in prepared houses on the inter-row distance from 70 cm and distance in the row from 40 cm. Previously, in autumn 2015 done deeply plowing and added 500 kg ha^{-1} of inorganic mineral fertilizers – improvement of soil "Protect Forte". That's right, the optimal soil pH is achieved, given that this plant looking for carbonate soil and active CaO. Before the spring sowing soil preparation was carried out in March. In 2017 was done manually mowing - cutting with special scissors (Figure 1). The yield of the fresh biomass of was determined by measuring the total biomass individual bush with each experimental plot. Biomass of raw materials, measured by a technical scale. The same day raw material is transported to a distillery, where is the method of distillation water vapor obtained essential oil (Miloradović et al., 2018).

Statistical analysis. Yield of H. arenarium, content of essential oil and plant height of vegetation were characterized using the basic statistics: mean, standard deviation, minimum, maximum, and coefficient of variation. The significance of differences between locations was analyzed using LSD tests.

Soil conditions. At the site in Kacarevo (Municipality Pancevo) the experiment is based on soil - carbonate chernozem (pH in KCL was 5,75; pH in H_2O was 6.95%, Total N 1.19%; K_2O and P_2O was 23.80-19.51 mg $100g^{-1}$; and $CaCO_3$-1.42%) while at the locality of Lešje (Municipality of Paracin) appointed the brown forest soil (pH u KCl 5,67; pH u H_2O 6.18%, Total N 1.13%; K_2O and P_2O 19.30 – 9.56 mg $100g^{-1}$; $CaCO_3$ – 0.64%).

Picture 1. Hand - cut harvesting yield crude biomass in the Kačarevo plot, Miloradović et al., 2018

Meteorological data. In Serbia, are highly variable meteorological conditions, both in terms of atmospheric precipitation and in terms of oscillations in air temperature (Popovic et al., 2015, 2018; Glamočlija et al., 2015; Stevanović et al., 2017, Terzic et al, 2017). The vegetation period 2017 average precipitation was 340.4 mm in Pančevo and 523 mm in Paraćin (Figure 1). In 2017, mean air temperature, in the southern Banat area were at the level of long-term average (12.6^{o}C) while they are in Pančevo were higher for 1,1oS (12.6^{o}C) multi-year average of Paracin.

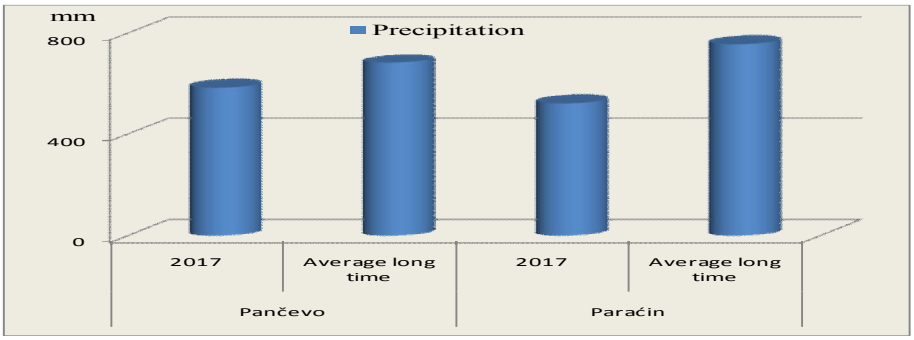

Figure 1. Quantity and distribution of precipitation in the area of Serbia, 2017

RESULTS AND DISCUSSION

Plant height at the stage of flowering (technological maturity).

The measurements were made immediately before manual mowing the flower. Average plant height in the period of technological maturity was 40.8 cm. On the experimental field in Kačarevo average plant height seaside plants immortelle in this

phenol-phase was 39.4 cm, and Lešje 42.2 cm or 7.3% higher but these variations were not significant (Table 1 and Figure 2).

Tabela 1. Anova for tested parameters

Parameter	D. of Freed.	SS	MS	F	p
Plant height, 10.7.2017.					
Intercept	1	19943.05	19943.05	3223.554	0.000000
Locality	1	23.52	23.52	3.802	0.079775
Error	10	61.87	6.19		
Total	11	85.39			
Yield of crude biomass per plant					
Intercept	1	650722.6	650722.6	1052.381	0.000000
Locality	1	6102.0	6102.0	9.869	0.010485
Error	10	6183.3	618.3		
Total	11	12285.4			
Essential oil content in dry herb					
Intercept	1	26.91008	26.91008	145.7316	0.000000
Locality	1	0.46808	0.46808	2.5349	0.142441
Error	10	1.84655	0.18466		
Total	11	2.31463			

Table 2. Descriptive statistics for tested parameters

Parameter	Locality	N	Average	Std. Dev.	Std.Err	-95,00%	+95,00%
Plant height, 10.7.2017.							
Average		12	40.766	2.786	0.804	38.996	42.537
Locality	Kačarevo	6	39.366	2.737	1.118	36.494	42.239
	Lešje	6	42.167	2.208	0.902	39.849	44.485
Yield of crude biomass per plants							
Average		12	232.867	33.419	9.647	211.633	254.100
Locality	Kačarevo	6	210.317	19.353	7.901	190.007	230.626
	Lešje	6	255.417	29.362	11.987	224.603	286.230
Essential oil content in dry herb							
Average		12	1.497	0.458	0.132	1.206	1.7889
Locality	Kačarevo	6	1.695	0.410	0.167	1.265	2.125
	Lešje	6	1.300	0.448	0.183	0.829	1.771

Parameter		PH	YCB per plants	EOC in dry herb
LSD	**0.5**	3.199	31.986	0.553
	0.1	4.551	45.496	0.786

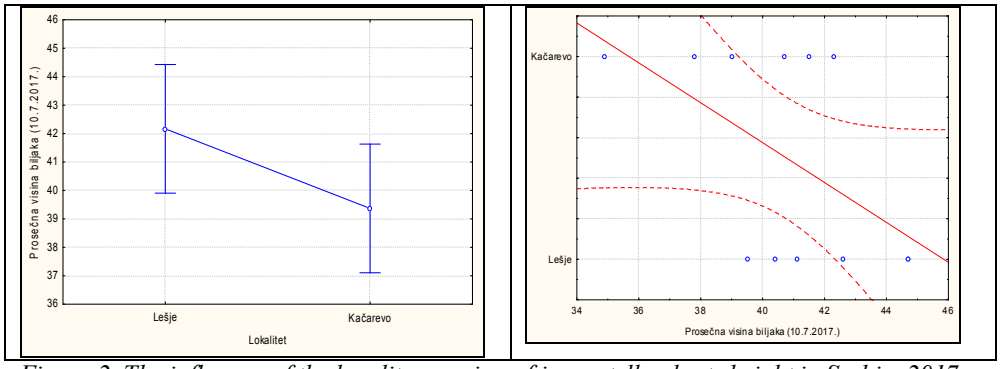

Figure 2. The influence of the locality growing of immortelle plants height in Serbia, 2017

In the first half of 2017 weather conditions they were favorable for the winter cereal crops, as well as for species that they're getting over in technological maturity during June. In such meteorological conditions, coastal immortelle has formed a significant one vegetative biomass and a large number of floral stems, so that it is obtained by a high herb yield. The average yield of fresh herbs per plant, for all elementary plots was 232.9 grams. On experimental plots in Kačarevo yield of fresh weight per plant was 210.3 g, and in Lešje by 21.4% higher (Table 1, Figure 3).

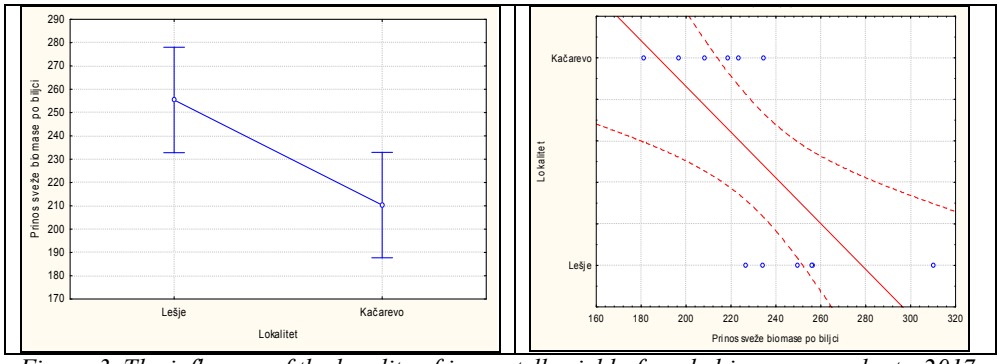

Figure 3. The influence of the locality of immortelle yield of crude biomass per plants, 2017

The content of essential oil in a dry herb, was performed on an average sample of the plant material, taken from both locations. The average essential oil content of herbs immortelle, for all localities was 1.5%. Thermal conditions, precipitation as well as the locality growing influenced the higher content of essential oil at locality of Lešje. Analyzes have shown that the dry herbs is a immortelle had more essential oils at Lešje for 31% (Table 2). At the locality of Lešje, average temperatures they were higher by 0.40°C, while total precipitation

were lower by 61 mm, in comparison with locality Kačarevo which affected the higher content of essential oil, Figure 1 and 4.

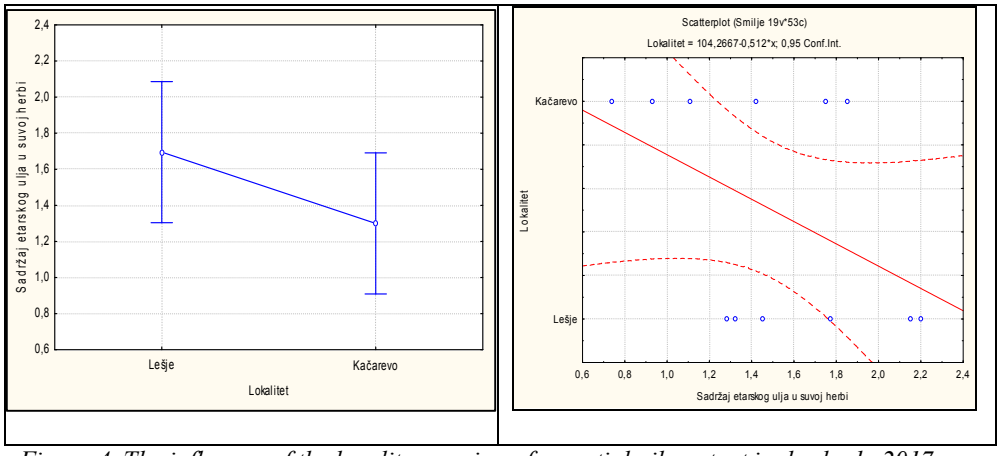

Figure 4. The influence of the locality growing of essential oil content in dry herb, 2017

Quantity and distribution of rainfall during the vegetation season,affects the quantity and chemical composition of essential oils as well as thermal conditions, as stated Tundis et al. (2010) which research carried out on two locality, the island of Sardinia and the southern Italian province of Calabria.

Immortelle can be cultivated by direct sowing which is the hard way and rarely applied. Another way is cultivated over seedling which is produced by sowing seeds in a protected area (leaf, greenhouse or greenhouse) during August (Sawilska and Jendrzejczak, 2013). It's the third way planting cutters cut dividing tillers and is applied mainly in selection and seed production, and much less in commercial production. The seedlings on open field is when the soil is heated at 10-15^{o}C. Seedlings amounts to open field, when it soil heated to 10-15^{o}C. In continental areas that's in the second half of April. Planting density depends on morphological traits of the species (cultivars), soil conditions and method of harvesting. The best plant layout on fertile soil is obtained if they are replanted at a distance of 70 cm x 40 cm. At this distance planted plants provided density of crops of about 35,000 plants per hectare. After replanted plants need watered and add NPK mineral nutrients, and after few days fill in the places where seedlings not received. Timely and professional pruning in the coming seasons will increase the lushness of plants and quality of crops, and these are elements which determine the planned yield. Drying the herbs immortelle runs on networks with wooden frames in a thin layer to turn it off need for rolling over biomass. In drying rooms it is necessary to provide quality air circulation. Official drug immortelle is a dried flower (*Helichrysi italici* flos), which serves for the

distillation of essential oil (*Helichrysi italici* aetheroleum) so that by cutting are cut only flourish with peak parts of stems. It's part of the stem above the top leaves. If the mowing was lower, to woody parts of the bush, that would have prevented further growth of the plant. From 2.5 kg of fresh biomass obtained 1 kg naturally dry. Dried drugs it is packed with appropriate packaging, and those are cardboard boxes or bags and keeps it in warehouses for Medicinal Herbs. The essential oil is kept on dark place at a temperature of up to 20 ° C in, to the top filled glass bottles volume 1-5 liters or aluminum canisters volume 1-100 liters. Dishes in which the oil is kept vacuum are closed to prevent it polymerization processes and loss of quality. On larger agricultural areas immortelle was mowed mechanically, special or customized machines, while on smaller fields mostly performed manually. In the first year it was obtained one mowing biomass, harvest was early November. Fresh biomass yield was 2.398 kg ha^{-1} while in the second year harvest carried out in mid-July, and the average yield of fresh biomass was 5.640 kg ha^{-1} (Miloradović, 2018). The yields of fresh flowers in full maturity of crops are in the range of 7-8 t ha^{-1}. Drying of crude biomass obtained 3.5-4 t ha^{-1} dry flowers (Jade Shutes, 2006; Gavarić et al., 2017).

Crop immortelle it can be used for about 10 years. First economic results they get in the second year when expected from 0.2 kilograms per plant. In the third year average yield per plant are 0.4 kilograms, when production it becomes economically justified. From the fourth to the eighth year yields are about 0.45 kilograms. Later, yields of herbs are falling, at 0.3 kilograms per plant. Maintenance costs and harvesting one hectare of crops at the annual level are maximum up to 1,400 euros (Miloradov, 2018).

Essential oils besides its medicinal properties, can also be used as bioherbicides. Research, carried out by Matković et al. (2017) have shown that essential oils three aromatic species (*Anethum graveolens, Juniperus communis* and *Salvia officinalis*) have shown an inhibitory effect on germination and seeding of seeds two species allergenic weeds (*Ambrosia* sp). Arenarin is a resinous, fragrant extract obtained from essential oil immortelle and represents a complex substance which exhibits antibacterial effect. By applying certain essential oils encourages the antibacterial activity against certain pathogenic bacteria *Staphilococcus aureus* and *Escherichia coli.*

CONCLUSION

The obtained results show that agricultural areas were tested in Republic of Serbia suitable for growing seaside immortelle, both by weather and by land conditions. However, in determining growing areas, priority should be given mountain areas and poorer land.

Plants of immortelle adopt small amounts of NPK assimilation, so it is forest soil represented good land for successful coastal production of immortelle.

Essential oil content in a dry herb was significantly higher in plants from the Lešje locality.

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ПРИНОС СВЕЖЕ БИОМАСЕ СМИЉА И САДРЖАЈ ЕТАРСКОГ УЉА У ЗАВИСНОСТИ ОД ЛОКАЛИТЕТА ГАЈЕЊА

Сажетак

Експериментални огледи са смиљем изведени су на два локалитета: Качарево (44°57′59″N 20°41′22″E, општина Панчево) и Лешје (општина Параћин, 43°51′10″N

20°41′22″E, Поморавље), на два типа земљишта (чернозем и гајњача). Потребе за квалитетним етарским уљем смиља у свету расту, како се оно све више користи у многобројним гранама индустријске прераде. Укупан принос свеже биомасе и садржај етарског уља био је у границама максималне продукције која у ове биљне врсте почиње од треће године и траје око 10 година. Добијени почетни резултати показали су да италијанско смиље може da се успешно гаји и у континенталним пределима у брдском подручју Централне Србије, као и у равничарским пределима Војводине.

Кључне речи: италијанско смиље, агроеколошки услови, принос свеже хербе, садржај етарског уља, Србија.