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Article

The First Study on Cultivating Roman Chamomile (*Chamaemelum nobile* (L.) All.) for Its Flower and Essential Oil in Southeast Serbia

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Abstract: Roman chamomile (*Chamaemelum nobile* (L.) All.) is a perennial herbaceous medicinal plant species that has not yet been thoroughly researched in terms of the influence of growing conditions on its morphological characteristics, flower yield, and the content and quality of its essential oil (EO). The plant material was harvested in the subsequent two production years at three localities in Southeast Serbia, differing in soil type (Alluvial soil, Rendzina, and Calcomelanosol). Based on two-year average values, the best results were obtained from plants grown on Rendzina, including the yield of fresh flowers (1850.2 g/m^2), the highest plant height (49.3 cm), the number of branches (4.1), leaves (11.6), and flower heads (3.6), the flower diameter (1.6 cm), and the essential oil content (1.6%). The major EO constituent obtained from the plants growing on Rendzina and Calcomelanosol was 3-methyl pentyl angelate (20.8% and 15.2%, respectively). In the EO obtained from the plants growing on Alluvial soil, the major EO constituent was isobutyl angelate (13.0%), while the content of 3-methyl pentyl angelate was close to it (12.2%). The outcomes of this study provide guidelines for further research related to the cultivation of a Roman chamomile genotype on various soil types in a hilly region of Southeast Serbia, where most cultivated plants cannot be grown.

Keywords: *Chamaemelum nobile* L.; soil types; flower yield; essential oil yield and quality

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1. Introduction

Roman chamomile (*Chamaemelum nobile* (L.) All., syn. *Anthemis nobilis* L.) is a perennial herbaceous species of the Asteraceae family. In nature, it is widespread throughout Europe, North Africa, and Southwest Asia. Nowadays, it is cultivated in several countries, including Belgium, France, Italy, Germany, England, Egypt, Algeria, Hungary, Poland, Bulgaria, and Argentina [1,2]. In Serbia, it spontaneously grows in the southeast part of the country (Figure 1).

Roman chamomile is a versatile plant that can be used both fresh and dried for culinary uses (it flavors soups, stews, and salads) in addition to its well-known medicinal purposes (it promotes relaxation, aids digestion, relieves anxiety, and soothes skin irritations) [3].

The aerial part of flowering plants is commonly used for extracting essential oil (EO). Fresh flowers yield around 1% of the oil, while dry ones yield 1.6% [4]. According to European Pharmacopoeia, the flowers must contain not less than 7 mL/kg of the oil [5]. The oil is a highly aromatic, clear liquid with variable colors: bluish when freshly distilled from fresh flowers and yellowish-green after only a few days. If distilled from the dried flowers, the oil is greenish-yellow. It is rich in esters (mainly angelic acid and tiglic acid esters) and many other compounds, including chamazulene, farnesene, α-bisabolol, bisabolol oxides A and B, and apigenin, which all contribute to the overall therapeutic properties of the plant [4]. In addition to the EO, the flower also contains coumarins (scopoletin-7-glucoside, herniarin, and umbelliferone), flavonoids (apigenin, luteolin, patulin, quercetin, etc.), anthemic acid, phenolic acid, fatty acids, phytosterols, choline, inositol, and others [6]. In vitro experiments with the Roman chamomile flower or its EO demonstrated antimicrobial, antiplatelet, and anti-inflammatory effects, while in vivo experiments on animals with the flower's EO and extracts showed anti-inflammatory, diuretic, and hypoglycemic properties [7]. Due to its calming and relaxing properties for both the mind and body, the oil of Roman chamomile is frequently used in aromatherapy, whether diffused in the air or added to bathwater or massage oils [7]. As it is also valued for its soothing and anti-inflammatory properties (calms dry, sensitive, or irritated skin and reduces redness and inflammation), it is a popular ingredient in many skin and hair care products, such as creams, serums, soaps, shampoos, and balsams [8,9]. The oil also found its application in agriculture as an important constituent of biopesticides; its aqueous extract is said to inhibit several stored products fungi, including *Aspergillus candidus*, *A. niger*, *Penicillium* sp., and *Fusarium culmorum* [10].

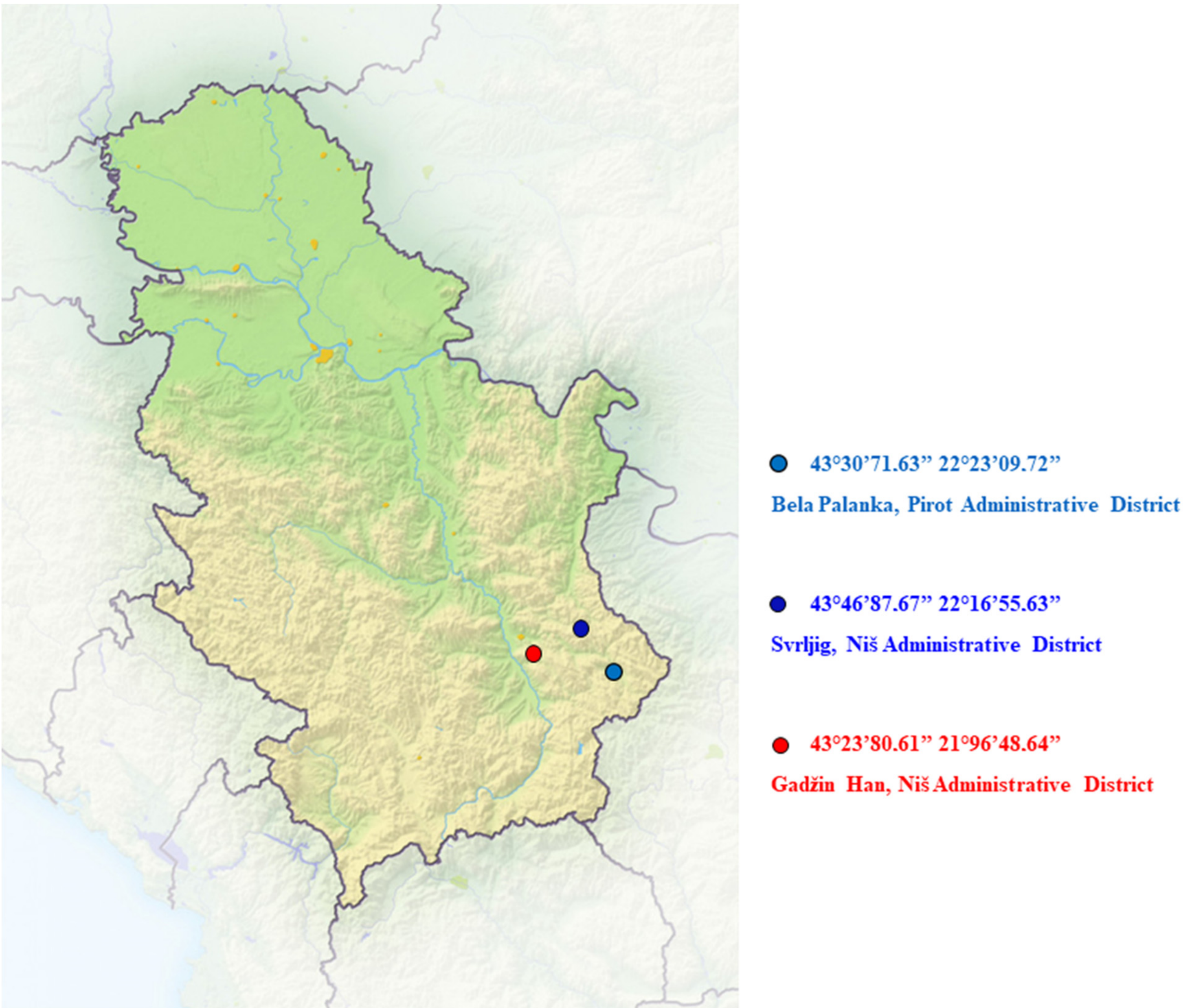

Figure 1. Localities in Southeast Serbia where Roman chamomile was experimentally cultivated for the purpose of this study.

Apart from being cultivated mainly for its flowers, Roman chamomile is also grown to create a fragrant ground cover in many parks and gardens. Although the scientific literature on the cultivation of Roman chamomile for its EO is rather scarce, certain data suggest that about fifty years ago, the annual production of this oil rarely exceeded 500 kg [11,12]. Roman chamomile has an economic cultivation period of 3 to 4 years [13]. It grows both on saline and structurally rich [6] and fertile soils [14], prefers sunny to partial shade positions, and although it is drought tolerant, adequate and consistent moisture levels are essential for Roman chamomile plants to produce high-quality flowers rich in EOs. As it grows quickly, without any particular need for feeding, in some locations, it is considered invasive. However, when cultivated, it achieves a better yield and quality of both the flowers and the EOs [15].

In the Republic of Serbia, there have been no previous studies on the cultivation of Roman chamomile. Therefore, the aim of this study was to determine, through field and laboratory tests, the influence of soil type and climate on the morphological and productive traits of cultivated Roman chamomile and the content and quality of its EO.

2. Materials and Methods

2.1. Localities with Experimental Cultivations of Roman Chamomile

The experimental cultivation of Roman chamomile was established at three localities in southeast Serbia, as described in Table 1.

Table 1. Data on the locality, soil type, and position where Roman chamomile experimental cultivations were established.

Locality	Soil Type	Altitude (m)	Latitude (°N)	Longitude (°E)
Gadžin Han	Alluvial soil	230	43°23′80.61″	21°96′48.64″
Svrljig	Rendzina	400	43°46′87.67″	22°16′55.63″
Bela Palanka	Calcomelanosol	270	43°30′71.63″	22°23′09.72″

Cuttings for research were taken from the Roman chamomile mother plants, which were grown and multiplied in the production unit of the Institute for Medicinal Plants Research "dr Josif Pančić" located in Pančevo (44°52′20.0″ N; 20°42′04.7″ E), South Banat, Republic of Serbia. Cuttings 10–12 cm long were taken by hand from three-year-old plants at the stage before buds appeared. One-year-old plants were investigated in the experiments. In early April 2022 and 2023, the cuttings of Roman chamomile were manually planted at a depth of 6–8 cm, at an inter-row distance of 80 cm, and in a row distance of 30 cm (i.e., 4.2 plants per m^2), which allowed for inter-row care measures (filling empty places, hoeing, and weeding). The trials were set as a completely randomized block system with four replicates; the basic plot size was 3.2 m^2 (3.2 m $\times$ 1 m). The identification of the studied plant species was previously confirmed by the author, Filipović Vladimir, and a voucher specimen was deposited at the herbarium of the Institute (IMPR 3-121). Chamomile flowers are large and white and made up of a solid conical receptacle.

For studied experimental cultivations, data on total precipitation (mm) and average monthly air temperature (°C) for the cultivation period of Roman chamomile were obtained from the nearest meteorological stations (MS Niš and MS Pirot). They were evaluated and compared to corresponding forty-year averages (1981–2010) for the Republic of Serbia.

2.2. Soil Analyses

Before setting up the experimental cultivations with Roman chamomile, the soil type was determined, which is presented in Table 1. For each of the soil types, the following analyses were performed: (1) the soil's pH in H_2O and KCl was determined in a suspension (10 g per 25 cm^3) using a potentiometer; (2) the total content of $CaCO_3$ was determined using the volumetric method with a Scheibler calcimeter; (3) the content of humus was measured using the modified Tyurin method; (4) the total N content was determined using the modified Kjedahl method after destruction in H_2SO_4; (5) the available P_2O_5 content was measured using a colorimeter after extraction with Al solution; and (6) the available K_2O

was measured using a flame photometer after extraction with an Al solution. Blanks and triplicate measurements were routinely included, and they were presented as arithmetic means of duplicate samples [16,17].

2.3. Morphological and Productive Traits

Prior to harvest, at each of the experimental cultivations, the following morphological traits were measured on 30 plants using a ruler or graph paper: plant height (cm), number of branches, leaves, and flower heads, as well as diameter (cm) and weight (g) of flower heads. Productive traits of Roman chamomile included measuring the fresh flower yields (g/m^2) at harvest and the dry flower yields (g/m^2) following the drying procedure.

2.4. Plant Material

In the two subsequent production years, 2022 and 2023, plant material was harvested from each locality where Roman chamomile was experimentally grown. The harvests were performed manually at the beginning of July (on a dry, sunny morning) when flower heads were in a phase of technological maturity. Following the harvest and measuring the obtained fresh flower yields, the plant material was left in a dryer at 40 °C to decrease moisture up to 11%, and then it was measured once again to obtain data on air-dry flower yields.

2.5. Essential oil (EO) Extraction Procedure

The EO oil isolation was performed using the hydro-distillation method by a Clevenger-type apparatus according to procedure I, described in the European Pharmacopoeia [5]. In short, 20 g of fresh flower heads were placed in a round-bottom flask (1000 mL) and filled with 400 mL of tap water. Distilled water was used as the distillation liquid in the graduated tube. The distillation lasted for 2 h after the boiling began. After the distillation was finished, the oil and water were left to settle for about 30 min, after which the oil was washed out of the graduated tube with 1 mL of hexane. The hexane was first evaporated, after which the collected oil was dried over anhydrous sodium sulfate to eliminate the residual water. The oil yield was expressed in percentages.

Sodium sulfate anhydrous (Na_2SO_4) and hexadecane were purchased from Sigma Chemicals Co. (St. Louis, MO, USA), while n-hexane was purchased from Zorka Pharma, Šabac (Serbia).

2.6. Essential Oil Sample Preparation and Chemical Analysis

Oil samples (20 µL) were dissolved in n-hexane (1.8 mL) and stored at 4 °C until further analysis.

The chemical composition of the EO was analyzed using the GC/MS technique. GC/MS analyses were performed on a Shimadzu GCMSQP2010 ultra mass spectrometer fitted with a flame ionic detector and coupled with a GC2010 gas chromatograph. The InertCap5 capillary column (60.0 m × 0.25 mm × 0.25 mm) was used for separation. Helium (He), at a split ratio of 1:5 and a linear velocity of 35.2 cm/s, was used as a carrier gas. Initially, the oven temperature was 60 °C, which was held for 4 min, then increased to 280 °C at a rate of 4 °C/min, and held for 10 min. The injector and detector temperatures were 250 °C and 300 °C, respectively. The ion source temperature was 200 °C. The identification of individual EO constituents was accomplished by comparing their MS spectra to those available from MS libraries (NIST/Wiley) and by comparing their experimentally determined retention indices (calibrated AMDIS) to data from the literature [18]. The EO composition was calculated based on the area of the obtained peaks and was expressed as a percentage.

2.7. Statistical Analysis

Data obtained in this study were statistically processed by a two-way factorial analysis of variance (a two-way ANOVA) using the program Origin Pro 9 and significance ratings derived from F-test and LSD-test for a significance threshold of 5%.

3. Results and Discussion

When Roman chamomile is cultivated for the production of its flower or EO, various practices may be implemented to maximize its yield and quality, including the selection of the proper variety, optimal growing conditions (plant density, fertilization and nutrient management, soil type, climate, etc.), and the proper harvesting time. In this study, the experimental fields were established with cuttings that derive from the spontaneous resources of southeast Serbia, which have never been studied nor cultivated before. Indeed, a Roman chamomile genotype was examined for its suitability for growing not only for its flowers but also for its EO. One of the limitations of this study is the lack of previous research regarding the cultivation of Roman chamomile not only in the Republic of Serbia but also in the wider region. In this regard, there were no data that could, to a certain extent, be the basis for the discussion of the results obtained in this study.

3.1. Meteorological Conditions during the Research Period

Precipitations and average monthly temperatures during the vegetation period of Roman chamomile (April–July) at three localities with experimental cultivations of Roman chamomile are presented in Table 2; the localities are grouped as those belonging to administrative districts Niš (Gadžin Han and Svrljig) and Pirot (Bela Palanka). From the presented data, it can be observed that there was a 24% decrease in total precipitation at localities Gadžin Han and Svrljig during the first vegetative period of Roman chamomile (2022), compared to the second one (2023). Also, in the first vegetation, for the mentioned two localities, the precipitation was significantly below the multi-year average, but the amount and distribution in the second one positively influenced morphological and productive traits and EO production, which will be further presented. Likewise, lower average temperatures in the second vegetative period at all localities, compared to the first one and to the multi-year average, positively influenced the yields of Roman chamomile flowers and their EO content.

Table 2. Precipitations (mm) and average monthly temperatures (°C) at localities with experimental cultivations of Roman chamomile in Southeast Serbia.

	Administrative District Niš Localities Gadžin Han and Svrljig				Administrative District Pirot Locality Bela Palanka				Republic of Serbia	
Month	Precipitations (mm)		T (°C)		Precipitations (mm)		T (°C)		Precipitations (mm)	T (°C)
	2021/22	2022/23	2021/22	2022/23	2021/22	2022/23	2021/22	2022/23	1981–2010	
XI	45.6	87.1	9.3	9.8	26.2	79.5	7.2	8.2	58.3	5.2
XII	86.6	64.8	4.2	6.5	93.9	58.0	0.4	4.1	55.3	0.8
I	22.0	76.0	1.5	5.6	43.3	26.0	−1.3	3.1	43.4	−0.4
II	15.6	19.6	6.1	4.7	38.8	12.5	3.1	1.2	41.1	1.0
III	6.7	58.6	5.7	9.4	28.6	13.9	2.2	6.3	46.8	5.5
Sum Average	176.5	306.1	5.4	7.2	230.8	189.9	2.3	4.6	244.9	2.4

Month	Precipitations (mm)		T (°C)		Precipitations (mm)		T (°C)		Precipitations (mm)	T (°C)
	2022	2023	2022	2023	2022	2023	2022	2023	1981–2010	
IV	58.1	40.9	11.9	10.0	24.4	29.8	10.4	8.64	56.7	10.7
V	23.0	41.9	19.0	16.0	72.4	52.6	16.9	14.5	64.9	15.9
VI	43.5	99.1	22.9	20.0	79.2	102.0	20.2	18.4	82.8	19.0
VII	58.6	59.6	24.4	24.0	61.8	15.2	21.8	23.2	63.5	21.0
Sum Average	183.2	241.5	19.6	17.5	237.8	199.6	17.3	16.2	267.9	14.9

3.2. Agrochemical Properties of Soil

The agrochemical soil properties of all localities under the experimental cultivation of Roman chamomile are presented in Table 3.

Table 3. Agrochemical analysis of the soil.

Soil Type (Locality)	pH		$CaCO_3$ (%)	Humus (%)	Total N (%)	Available (mg/100 g Soil)	
	in H_2O	in KCl				P_2O_5	K_2O
Alluvial soil (Gadžin Han)	7.88	7.15	6.36	2.32	0.117	7.6	12.9
Rendzina (Svrljig)	7.58	6.73	1.74	4.78	0.302	4.7	18.3
Calcomelanosol (Bela Palanka)	6.35	5.74	5.87	7.46	0.469	1.6	12.8

The highest pH value, both in H_2O and in KCl, was recorded in Alluvial soil (7.88 and 7.15., respectively). The content of $CaCO_3$ widely ranged from 1.74% in Rendzina to 6.36% in Alluvial soil. Humus content also varied, with Alluvial soil being the lowest (2.32%), while the soil types Rendzina (4.78%) and Calcomelanosol (7.46%) were humus-rich and humus-very rich, respectively, which is in agreement with some other studies [19,20]. The contents of available P_2O_5 in all soils were up to 7.6 mg/100 g, the highest in Alluvial soil, while the contents of available K_2O were up to 18.3 mg, being the highest in Rendzina.

A positive feature of this plant species is its great heterogeneity when choosing the type of soil for its growth. Some varieties prefer dry, sandy soils, while others require richer soils with a pH value of 6.5 to 8.0. However, excessively firm, water-bearing areas and loose sands are never recommended [13]. Based on the results obtained in this study (Tables 4–6), it is obvious that the examined genotype of Roman chamomile may be successfully cultivated on less fertile soils, as well.

3.3. Morphological and Productive Traits of Roman Chamomile

The results of the morphological and productive traits of Roman chamomile are shown in Table 4.

3.3.1. Influence of Year

Regarding the morphological traits, the influence of the year was observed in several traits, including plant height, number of branches, diameter, and weight of fresh flower heads. The highest plants were those from Svrljig (49.8 cm), and they were similar to the plants from Bela Palanka but significantly higher than those from Gadžin Han. The highest number of branches (4.1) and diameter of fresh flower heads (1.6 cm) were also recorded in plants from Svrljig, and both averages were significantly higher than the corresponding average values of the other two localities. Identical fresh flower average weights were observed in Svrljig and Gadžin Han (0.08 g), both significantly higher than the one in Bela Palanka. Regarding the productive traits, the influence of the year on dry yields differed between localities, the highest being obtained in Svrljig (1314.1 g/m^2); the yields in Gadžin Han and Bela Palanka were 1.55 and 3.4 times lower than those from Svrljig, respectively (Table 4).

Table 4. Influence of year, soil type, and their interaction on morphological and productive traits of Roman chamomile grown on different localities in Southeast Serbia; v.r.—values ranges; F pr.—the *p*-value, also referred to as the probability value or observed significance level; LSD $_{0.05}$—least significant difference at a significance level of 0.05.

Year	Soil Type	Locality	Plant Height (cm)	Number			Fresh Flower Heads		Yield (g/m^2)	
				Branches	Leaves	Flowers	Diameter (cm)	Weight (g)	Fresh	Dry
2022	Alluvial	Gadžin Han	38.0	1.5	9.0	2.8	1.3	0.05	1578.7	890.1
	Rendzina	Svrljig	49.8	2.8	10.5	2.3	1.6	0.05	1736.8	1314.1
	Calcomelanosol	Bela Palanka	46.3	3.0	12.0	3.3	1.5	0.07	485.5	319.3
2023	Alluvial	Gadžin Han	41.3	2.8	12.8	3.8	1.5	0.11	1624.5	791.9
	Rendzina	Svrljig	48.8	5.5	12.8	5.0	1.7	0.12	1963.5	1289.9
	Calcomelanosol	Bela Palanka	46.3	3.0	12.0	3.3	1.5	0.07	1014.7	447.7
Average	Alluvial	Gadžin Han	39.6 ^{c}	2.1 ^{b}	10.9 ^{a}	3.3 ^{a}	1.4	0.08 ^{a}	1601.6	841.0 ^{b}
	Rendzina	Svrljig	49.3 ^{a}	4.1 ^{a}	11.6 ^{a}	3.6 ^{a}	1.6	0.08 ^{a}	1850.2	1302.0 ^{a}
	Calcomelanosol	Bela Palanka	46.3 ^{b}	3.0 ^{ab}	12.0 ^{a}	3.3 ^{a}	1.5	0.07 ^{a}	750.1	383.5 ^{c}
Years		v.r.	0.08	6.15	11.40	6.04	0.85	45.85		29.22
		F pr.	0.779	0.025	0.004	0.027	0.372	<0.001		<0.001
		LSD $_{0.05}$	5.60	1.14	1.42	1.08	0.17	0.01	105.4	82.4
Soil type		v.r.	4.68	4.64	7.62	0.24	3.52	3.33		181.63
		F pr.	0.026	0.027	0.005	0.788	0.056	0.064	<0.001	<0.001
		LSD $_{0.05}$	6.86	1.40	1.74	1.33	0.21	0.01	129.0	100.9
Interaction		v.r.	0.24	2.19	3.80	2.50	0.28	12.11		8.14
		F pr.	0.791	0.147	0.046	0.116	0.758	<0.001	0.004	0.081
		LSD $_{0.05}$	9.70	1.98	2.46	1.88	0.30	0.02	182.5	142.7

3.3.2. Influence of Soil Type

Regarding the morphological traits, the influence of soil was observed in the following traits: plant height, number of branches, and weight of fresh flower heads. The highest in all three morphological traits were the plants growing on Rendzina soil, except the plants growing on Alluvial soil, which showed an identical average flower weight. Regarding the productive traits, the influence of soil on the obtained dry yields was significant in all localities, but they differed (Table 4).

3.3.3. Influence of Interaction Year x Soil Type

Regarding the interactive influence of the year and the soil type, significant effects were observed between Roman chamomile plants cultivated in various experimental fields. Regarding the *morphological traits*, the highest and most branched plants were observed on Rendzina soil, which differed in the mentioned two traits only from plants grown on Alluvial soil. Regarding the *productive traits*, the yields of dry flowers differed between localities, the highest being observed at locality Svrljig.

To the best of our knowledge, the results on the morphological and productive traits of Roman chamomile grown under the agroecological conditions of three different localities in Southeast Serbia came out as the first study conducted in this part of Europe. Therefore, they could not be compared to any other study conducted in the neighboring region. In a study conducted in Iran (the province of Yazd), the average yield of dry Roman chamomile flowers was 1.089 g/m^2 [21], which is below the highest dry yield obtained at the locality Svrljig but higher than those obtained at the other two localities in Serbia (Table 4). Regardless of the localities, the number of plants in Serbia was higher than in the study conducted by Sharafzadeh and Alizadeh [6], where the plants were up to 30 cm high. Our findings support the earlier claim that the height of Roman chamomile plants ranges between 30 and 60 cm depending on the agroecological conditions under which the plants are grown [22]; the plants in our experiment were slightly shorter (38.0–49.8 cm), regardless of the production year. The harvesting of fresh flowers was performed at the stage of full blossom [23], in which 50% of the flowers are open, and the first ray of florets begins to fall (i.e., in the stage of technological maturity). According to FAO data [13], the yield of fresh Roman chamomile flowers in the first production year ranges between 4 and 6 t/ha, while in the following years, the interval range was between 10 and 12 t/ha. In this study, the yields of fresh flowers in the first year were between 4.9 and 17.4 t/ha, while in the second one, the interval range was between 10.1 and 19.6 t/ha, both years being the highest at locality Svrljig (Table 4). In the absence of other literature data, the aforementioned FAO's limited data are used only as a benchmark against which our findings are quantitatively compared, even though it is unclear exactly which region of the world they pertain to, how Roman chamomile was grown, or whether data on yields refer to a cultivar that is grown for the production of flowers of EO.

3.4. Content and Composition of Roman Chamomile Essential Oil (EO)

The contents of EOs of the flowers of Roman chamomile cultivated at three experimental localities in Southeast Serbia are presented in Table 5.

Table 5. The EO content in Roman chamomile cultivated at three experimental localities in Southeast Serbia (%).

		Essential Oil Content (%)			CV
Locality	**Soil Type**	**2022**	**2023**	**Average**	**(%)**
Gadžin Han	Alluvial soil	0.72 [b]	0.89 [b]	0.80 [b]	21.01
Svrljig	Rendzina	1.50 [a]	1.74 [a]	1.62 [a]	17.45
Bela Palanka	Calcomelanosol	0.68 [b]	0.80 [b]	0.74 [b]	10.17
	Average	0.97	1.14	1.05	16.21
	$LSD_{0.05}$	0.22	0.31	0.27	
	F-test	**	**	**	

In this study, the highest average content was achieved in plants cultivated on Rendzina soil (1.62%), which differed from the other two soil types, which did not differ between themselves. However, all the contents proved to be in agreement with the previous literature data, stating that they range between 0.22 and 1.9% [23–29]. According to the literature, the content of EO in Roman chamomile depends on plant development [30], ranging from 0.22% in intensive vegetative growth to 0.80% in the full flowering stage. Also, the deficit of moisture during the production of Roman chamomile may cause certain physiological disorders, such as a reduction in photosynthesis and transpiration [31], which may affect the content and composition of EO in any aromatic plant. Certain similarity was observed in this study; due to lower precipitations during the first vegetation of Roman chamomile (April–July 2022), the content of its EO was lower than in the corresponding period in the second production year (2023), which was much more favorable for the plants (Table 2). The obtained EO contents in 2023 were higher at all studied localities, which is attributed to more favorable climatic conditions that year, particularly during the period of the synthesis and accumulation of EO. In addition, the percentage of the EO content increase in 2023 compared to 2022 varied, depending on the locality, between 16.0 and 23.6%, the highest being observed in plants cultivated on Alluvial soil (Gadžin Han).

The chemical profiles of Roman chamomile EO samples for both production years and each studied locality are comparatively presented in Table 6.

Based on their retention times and mass spectrometric data, 31 compounds in total were determined in the EO samples obtained from Roman chamomile flowers produced at three localities differing in soil types. In all EO samples, regardless of the production year or locality, the examined EOs were primarily composed of monoterpenoids, and their average contribution to the total EO ranged between 51.95 and 87.8%. Among this chemical class, the oxygenated monoterpenoids prevailed (72.5–87.8%) over monoterpene hydrocarbons

(9.4–23.8%). The content of sesquiterpenoids (sesquiterpene hydrocarbons and oxygenated sesquiterpenes) showed to be almost negligible in the EOs, ranging between 0.1 and 1.2%.

Table 6. Chemical composition of Roman chamomile essential oils; RI_{lit}–retention indices from the available literature (Adams, NIST, Wiley); RI_{exp}—retention indices from this experiment.

			Contribution to Essential Oil (%. *w*/*w*)					
			Alluvial Soil Gadžin Han		Rendzina Svrljig		Calcomenasol Bela Palanka	
RI_{lit}	RI_{exp}	Components	2022	2023	2022	2023	2022	2023
869	873	Isopentyl acetate	0.5	0.4	0.3	0.4		
875	875	2-Methyl butyl acetate	0.2	0.5	0.2	0.3		0.3
908	912	Isobutyl isobutyrate	3.2	3.2	2.0	1.9	2.2	2.2
914	919	*3-methyl-2*-Buten-1-ol acetate	5.1	5.0	4.4	4.5	0.5	0.5
932	936	*α*–Pinene	10.5	10.9	4.7	4.7	6.0	6.1
946	952	Camphene	12.1	12.3	3.2	3.4	1.4	1.4
974	979	*β*–Pinene	1.0	0.7	1.5	1.2	0.7	0.9
988	991	Myrcene	1.1	1.0	1.3	1.3	0.8	0.8
1007	1009	Isoamyl isobutyrate	2.3	0.7	3.1	0.6	3.2	0.4
1045	1049	Isobutyl angelate	12.3	13.6	4.3	4.4	10.6	10.7
1048	1054	Prenyl isobutyrate	1.7	2.0	3.0	3.1	1.4	1.6
1056	1064	Artemisia ketone	15.9	4.1	10.7	11.4	8.8	9.0
1063	1070	*n*–Octanol			0.6	0.5	0.2	
1085	1086	Butyl angelate	0.6	0.6	0.4	0.4	0.4	0.4
1088	1089	Isobutyl tiglate	0.2		0.1			
1100	1101	2–Methyl butyl-2-methyl butyrate	0.5	0.4	0.9	0.9	1.1	1.1
1127	1133	Octyl formate	6.0	5.4	7.9	7.9	3.5	3.6
1143	1147	Isoamyl angelate	5.6	5.5	8.6	8.7	13.8	13.7
1148	1151	Isoamyl tiglate	5.5	5.6	6.3	6.4	11.4	11.2
1145	1156	Camphene hydrate		0.4			0.8	0.4
1145	1158	Myrcenone	1.3	0.8	0.7	0.8		0.5
1160	1169	Pinocarvone	1.4	1.1	4.0	3.9	8.7	8.5
1165	1173	Borneol	0.5		0.3		0.4	0.5
1189	1190	Prenyl angelate	3.4	3.0	4.7	4.7	3.2	3.2
1197	1201	Butanoic acid. 2-methyl-4-methylpentyl ester	0.5		2.0	2.4		
1195	1202	Myrtenal					2.6	3.1
1249	1251	3–Methyl pentyl angelate	4.9	19.1	20.7	20.9	15.2	15.1
1275	1276	*3Z*–Hexenyl angelate			0.3		0.1	
1417	1435	Caryophyllene(E-)					0.1	
1493	1497	*trans*–Muurola-4(14).5-diene	0.2		0.2		1.0	1.0
1627	1627	*1-epi*-Cubenol	0.4				0.2	
		Monoterpene hydrocarbons	23.7	23.9	9.4	9.4	8.2	8.4
		Oxygenated monoterpenes	72.7	72.3	86.8	85.1	88.9	86.7
		Sesquiterpene hydrocarbons	0.2	0.0	0.2	0.0	1.1	1.0
		Oxygenated sesquiterpenes	0.4	0.0	0.0	0.0	0.2	0.0
		Total identified	97.0	96.2	96.4	94.5	98.4	96.1

The analysis of the EO samples also revealed the following variability interval for the most dominant EO constituents, regardless of the soil type on which the plants were grown: 3–methyl pentyl angelate (4.9–20.9%) > artemisia ketone (4.1–15.9%) > isoamyl angelate (5.5–13.8%) > isobutyl angelate (4.3–13.6%) > camphene (1.4–12.3%) > isoamyl tiglate (5.5–11.4%) > α–pinene (4, 7–10.9%).

Regarding the influence of the examined soil types on the composition of Roman chamomile EOs, several differences in the content of major constituents were observed. Based on a two-year average value, in the EO samples deriving from Rendzina, the most abundant compound was 3–methyl pentyl angelate (20.8%), followed by artemisia ketone (11.1%) and isoamyl angelate (8.7%), which together amounted to 40.6% of the total EO. In EO samples from plants grown on Calcomelanosol, the major compound was also 3-methyl pentyl angelate (15.2%), followed by isoamyl angelate (13.8%) > isoamyl tiglate (11.3%) > isobutyl angelate (10.7%); their total share of the total EO was 50.9%. Although the soil type was not determined, in the EO samples from Lithuania, the major EO constituent was the same as in this study, 3-methylpentyl angelate (20.1–27.6%), but other dominant EO constituents differed in contribution to the total EO and they were as follows: methallyl angelate (7.3–10.3%) > isoamyl angelate (5.57–9.02%) > isobutyl angelate (4.8–6.8%) [30,32].

In the EO samples from Roman chamomile grown on Alluvial soil, two-year average values showed that the most abundant compound was isobutyl angelate (12.95%), followed by camphene (12.20%) and 3-methyl pentyl angelate (12.2%), which, together, amounted to 37.3% of the total EO. In several other studies on Roman chamomile EO composition, isobutyl angelate was also reported as a major constituent. Ranging from 15.7 to 44.8%, while the contribution of the other main constituents differed from the ones observed in our study: 2-methyl-2-propenyl angelate (12.1–12.5%) [33,34] > 2-methylbutyl angelate (6.6–20.3%) [25,33,35–37] > propyl tiglate (10.8–13.1%) [25] > isoamyl angelate (27.4%) [25,30,34,37] > amyl angelate (18.0%) [24] > 3-methylamyl angelate (7.8–11.3%) [34].

Apart from the mentioned literature data, which share our findings that the major constituents of Roman chamomile EO are either 3-methyl pentyl angelate or isobutyl angelate, sporadic reports are claiming different major constituents in their samples, including α- and β-pinenes [28,29], or methyl allyl angelate and 3-methyl pentyl angelate [33] or pentadecyl-3-methyl-2-butenoate and hexadecyl-3-methyl-2-butenoate [28].

3.5. Correlations among Variables

The relationship between two continuous variables* is calculated using the simple linear correlation coefficient, also called Pearson's correlation coefficient. The correlation matrix between the investigated parameters is presented in Table 7.

Table 7. Correlation matrix between the researched parameters.

Parameters		Dry Flower Weight (g/m^2)	Plant Height (cm)	Number of Branches	Number of Leaves	Number of Heads	Diameter of the Head (cm)	Head Mass (g)	Essential Oil Content (%)	3–Methyl Pentyl Angelate
Dry flower weight (g/m^2)	Pearson Corr.	1	0.10324	0.19063	0.39543	0.06092	0.13824	0.13949	0.77382 *	0.34533
	Sig.	--	0.63118	0.37225	0.0558	0.77734	0.51946	0.51564	9.18×10^{-6}	0.09838
Plant height (cm)	Pearson Corr.	0.10324	1	0.60262 *	0.38235	0.19124	0.55899 *	0.06729	0.42262 *	0.38038
	Sig.	0.63118	--	0.00183	0.0652	0.3707	0.00452	0.75472	0.03965	0.06671
Number of branches	Pearson Corr.	0.19063	0.60262 *	1	0.59464 *	0.40059	0.59318 *	0.43237 *	0.53974 *	0.48398 *
	Sig.	0.37225	0.00183	--	0.00218	0.0524	0.00225	0.03485	0.00649	0.01656
Number of leaves	Pearson Corr.	0.39543	0.38235	0.59464 *	1	0.49592 *	0.50794 *	0.51538 *	0.57743 *	0.75219 *
	Sig.	0.0558	0.0652	0.00218	--	0.01372	0.01128	0.00995	0.00313	2.24×10^{-5}
Number of heads	Pearson Corr.	0.06092	0.19124	0.40059	0.49592 *	1	0.24333	0.57502 *	0.24743	0.27496
	Sig.	0.77734	0.3707	0.0524	0.01372	--	0.25189	0.00329	0.24374	0.19348
Diameter of the head (cm)	Pearson Corr.	0.13824	0.55899 *	0.59318 *	0.50794 *	0.24333	1	0.37451	0.3579	0.45056 *
	Sig.	0.51946	0.00452	0.00225	0.01128	0.25189	--	0.07138	0.08595	0.02714
Head mass (g)	Pearson Corr.	0.13949	0.06729	0.43237 *	0.51538 *	0.57502 *	0.37451	1	0.33309	0.44416 *
	Sig.	0.51564	0.75472	0.03485	0.00995	0.00329	0.07138	--	0.11173	0.02968
Essential oil content (%)	Pearson Corr.	0.77382 *	0.42262 *	0.53974 *	0.57743 *	0.24743	0.3579	0.33309	1	0.64151 *
	Sig.	9.18×10^{-6}	0.03965	0.00649	0.00313	0.24374	0.08595	0.11173	--	7.29×10^{-4}
3–Methyl pentyl angelate	Pearson Corr.	0.34533	0.38038	0.48398 *	0.75219 *	0.27496	0.45056 *	0.44416 *	0.64151 *	1
	Sig.	0.09838	0.06671	0.01656	2.24×10^{-5}	0.19348	0.02714	0.02968	7.29×10^{-4}	--

2-tailed test of significance is used *-correlation is significant at the 0.05 level.

The strongest positive correlations were achieved between the following parameters: dry flower weight and EO content (r = 0.77382 *) > number of leaves and 3-methyl pentyl angelate (r = 0.75219 *) > EO content and 3-methyl pentyl angelate (r = 0.64151 *) > number of branches and plant height (r = 0.60262 *). In short, heavier flower heads indicate higher EO content, while more leaves suggest a higher content of 3-methyl pentyl angelate in the EO. Higher EO content implies higher 3-methyl pentyl angelate levels. All other correlations were not particularly pronounced (r < 0.60).

4. Conclusions

Due to favorable agroecological conditions, the hilly region of Southeast Serbia appears to be an attractive region for the cultivation of Roman chamomile. In the experiments, in two meteorologically different years, one-year-old plants were investigated on three types of soil. Two of the three soil types examined in the region had high humus content (4.8–7.5%), which positively affected the morphological and productive traits of cultivated plants. The plants growing in Svrljig (on Rendzina soil) were the highest (49.3 cm), which is considered a suitable feature for harvesting Roman chamomile flowers. They also achieved the highest dry flower yield (1302 g/m^2) and EO content (1.6%). Heavier flower heads of the examined Roman chamomile plants bear more EO, which contains more 3-methyl pentyl angelate, a characteristic constituent of this EO that is well-known for its pleasant smell and safe use in cosmetic products. This was a dominant compound in EO samples from Svrljig (Rendzina soil) and Bela Palanka (Calcomelanosol), contributing to the total EO of 15.2% and 20.8%, respectively. In EO samples from Gadžin Han (Alluvial soil), it accounted for 12.2% of the total oil and was the second most abundant compound, following isobutyl angelate (13.0%). The latter is a well-known fatty acid ester that is also commonly used in cosmetic products. According to the achieved results, the introduced new genotype of Roman chamomile can be successfully grown on all tested soil types in the hilly region of Southeast Serbia, where most cultivated plants cannot be grown.

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