

Organic vs conventional farming of oil-bearing rose: Effect on essential oil and antioxidant activity

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Abstract

The aim of this study was to establish whether the type of the agricultural system has any influence on the essential oil production and antioxidant activity of industrial cultivated *Rosa damascena* Mill. in the Rose valley, Bulgaria. Six private farms from Kazanlak (Rose) Valley, Southern Bulgaria were included in the study conducted in the period 2019–2020. The first three selected farms are designated within the conventional farming and the other three are certificated as organic farms. GC/FID and GC/MS analyses were performed; the contents of total polyphenols and flavonoids in the methanol extracts from rose petals were determined. Additionally, the antioxidant activity of rose extracts was evaluated by four reliable methods: 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid (ABTS), ferric reducing antioxidant power (FRAP), and cupric reducing antioxidant capacity (CUPRAC) assays. The impact of the agricultural system on the essential oil composition and antioxidant activity was evaluated by ANOVA statistical analysis. The results obtained showed that organic farming produced essential oil with a higher linalool and geraniol content and lower β -citronellol + nerol concentrations than conventional farming. It was found that organic farming production demonstrated a better antioxidant

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activity evaluated by the three DPPH, ABTS, and CUPRAC methods according to the averaged data for two years, 806.82, 797.66 and 1534.40 mM TE/g dw versus 510.34, 521.94 and 917.48 mM TE/g dw for CF, respectively, with high statistical significance for the DPPH and ABTS analyses. Consequentially, the rose extracts from the organic farming accumulated more phenolic compounds that corresponded to the higher antioxidant potential of the organic roses.

Keywords

cv. Raduga, DPPH, Rosa damascena Mill., rose oil composition, total phenol

Introduction

Globally, the oil-bearing rose, for the production of essential oil, is industrially grown mainly in Bulgaria, Turkey, Iran, India, Pakistan, China, Morocco, Egypt, France, and Russia. However, Bulgaria is the leading producer of the main genotype oil-bearing rose (Rosa damascena Mill.) (Gunes 2005; Kovacheva et al. 2010; Ucar et al. 2017). According to Gunes (2005), 100 kg fresh rose flowers are required to produce approximately 10 g of essential oil. The commercial cultivation of roses in Bulgaria predominantly includes R. damascena Mill. f. trigintipetala Dieck. (Kazanlak rose) due to its high rose oil content and chemical composition (Chalova et al. 2017). The observations of recent decades revealed that the cultivar Raduga has spread widely in Bulgarian rose plantations (Kovacheva et al. 2010). It is a cross pollination result of Rosa damascena Mill. X Rosa gallica L., with high productiveness and essential oil content. Recently, interest in rose oil production has been growing not only due to its perfuming effects but also because of the wide range of biochemical reactions, such as an analgesic, hypnotic, antispasmodic, anti-inflammatory and anticonvulsant, which the rose flowers exhibit. (Baydar and Baydar 2013; Kumar et al. 2013; Mahboubi 2016) On the other hand, there is a real interest in organic crop production, particularly with aromatic and medical plants. Organic agriculture is an alternative cultivation model involving the agricultural practice without chemical additives. This cultivation method is designed to encourage respect towards the biological cycles of the production system to maintain and to increase soil fertility, to minimise any form of pollution, to avoid the use of synthetic fertilizers and pesticides, to maintain the genetic diversity, to consider the wide social and ecological impact of the food production system and to produce good quality foods in sufficient quantities. (Giuseppina et al. 2011). The choice of organic production of an essential oil crop in comparison with the conventional one is frequently registered as expensive and more challenging for agricultural producers, but the results are of particular importance for improved lifestyle, health, and longevity. Thus, the production of organic rose flowers and oil requires the application of new agricultural practices, which require additional investments and technologies in order for the production to be certified as organic and cost effective. One of the main challenges in rose oil organic cultivation is the fact that R. damascena exhibits low disease resistance to major diseases and pests (Kovacheva et al. 2010). In this regard, the Integrated Pest Management (IPM) has a dominant role in organic rose oil cultivation. The most commonly applied IPM approaches include designing or redesigning the landscape, modifying the habitat to reduce the pest's resources and increase the habitats for natural predators, changing cultural methods such as cultivation, weeding, mulching as well as increasing inspections and tight monitoring of pest invasion (Chalova et al. 2017). Nunes and Miguel (2017) discussed the influence of several factors on the chemical composition of Damask rose oil and concluded that agricultural practices, important for essential oil productiveness and biochemical composition were mainly: method of propagation, time of the day of flower harvesting - air temperature, relative humidity, intensity of sunlight, flower stages, day period of harvesting, harvest procedures, time and level of pruning, storage of plant material, and method of distillation. Erdal and Munduz (2017) reported that the nutrient concentrations of the leaves from conventional gardens of *R. damascena* Mill were significantly higher than the organic ones, particularly for the nitrogen, manganese, and zinc content. Similarly, the flower nutrient concentrations of conventional gardens were higher for all examined nutrients, and the differences between organic and conventional gardens for the nitrogen, potassium, calcium, and iron concentrations were significant. The application of the anti-gibberellic, Paclobutrazol (PP333), combined with supplied nitrogen as NH₄-N and NO₃-N in appropriate amounts, and the micronutrients Mn^{2+,} Zn^{2+,} and Cu²⁺ enhanced the flower bud formation and flowering as well as the rose oil yield with higher percentage of citronellol. Ucar et al (2017) reported the significant effect of different irrigation amounts and nitrogen doses 160 kg ha⁻¹ and 240 kg ha⁻¹ on the flower yield and rose oil yield for 2011 and 2012 (P < 0.01), but it was not established to be significant for the first year of investigation 2010. The effect of the cultivation practices on the fruit quality and antioxidant capacity was evaluated in many studies. (Wang et al. 2008) There is a small number of studies investigating the effects of agricultural practices on the secondary metabolites in medicinal and aromatic plants (Malik et al. 2011). Our primary investigations showed that the highest values of total phenols and flavonoids are found in the rosewater extract from organically grown plants: 47.09±2.89 mg GAE/g dry weight and 6.87±3.00 mg QE/g dry weight, respectively (Petkova et al. 2020). The highest radical scavenging activity was demonstrated by the extracts from organic plantations, while the metal-reducing assays showed higher antioxidant potential in the extracts from conventionally grown roses. Since many of the bioactive secondary metabolites produced by medical plants have ecological roles in promoting plant survival under a range of environmental conditions (Briskin 2000), Malik et al (2011) confirmed that the production of secondary metabolites within the medicinal and aromatic plants is under diverse physiological, biochemical, metabolic and genetic regulations and can be manipulated by alterations in the growing conditions. The aim of our study was to continue the investigation in that field in order to answer clearly whether the type of the agricultural system has any influence on essential oil production and the antioxidant activity of industrial cultivated R. damascena in the Rose valley.

Materials and methods

Location and site description

The field study was conducted in private farms, located in the northwest part of the Kazanlak Rose valley, Bulgaria, in the two-year period of 2019–2020. The valley is situated at 400–500 m altitude, in the middle of the country between the Balkan range in the north and Sredna Gora mountain to the south. The climate is continental, the winters are generally cold and wet, and the summers cooler than in other parts of Bulgaria. January is the coldest month of the year with a -1.1 °C average temperature. July is the warmest month in Kazanlak with an average temperature of 21.2 °C. Spring in Kazanlak has the feel of winter, with late snowfalls. The annual precipitation rates range from 500 to 650 mm in the Kazanlak valley, with heaviest precipitation between April and June (Sobotkova and Ross 2018). Data about the climatic conditions of the studied years were provided from the local meteorological station, situated in Kazanlak. The monthly distribution of the average temperatures and rainfalls, compared to the 100-year average rate are presented in Figs 1, 2, respectively.

The field study was conducted in six private farms, as three of the oil rose private plantations are certified as organic farms whereas they apply an organic agricultural system and the rest of them are designated within the conventional farming. The farms are located close to each other and the dominant soil type is fluvisols. Fluvisols (Deluvial soils) are formed by downhill creep, where the sorting of materials comes about through gravity. Creep is the slow movement of soil masses down slopes that are

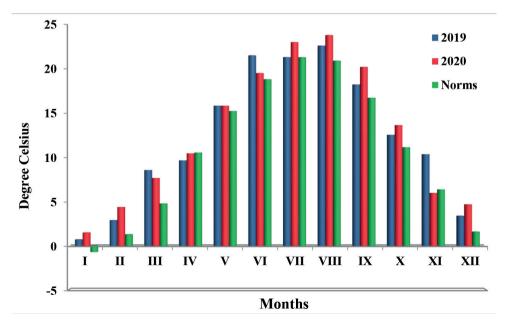


Figure 1. The average monthly temperatures (°C) for Kazanlak valley in 2019/2020.

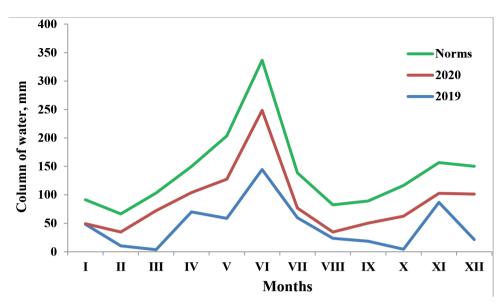


Figure 2. The average monthly rainfall (mm) for Kazanlak valley in 2019/2020.

usually steep. The process takes place in response to gravity where there is a pronounced water saturation (Shishkov and Kolev 2014). The soils in the region have ochric and nondiagnostic features, typical of fluvisols. The soil samples in all six arable areas were characterized by acid reaction, typical for that soil. The range of values of pH (H_2O) was between 4.20 and 6.10. The soil organic matter (OM) content varied between low and high content with values from 0.86 to 4.03%, where the mean OM, % value was 2.80. More detailed information about the soil characteristics was reported by Todorova et al. (2020).

Agricultural practices in the studied farms

Detailed characterization of the agricultural practices of the studied farms is presented in Table 1.

Farm 1 is characterized as typical conventional farming with soil tillage, mineral fertilization, foliar feeding with NPK + microelements during vegetation. The soil tillage included 3–4 hoeing with a cultivator between the rows.

Farms 2 and 3 are conventional with combined good agricultural practices, in our case - turf surface as mulching, drip irrigation, mineral fertilization, foliar feeding with NPK + microelements during vegetation.

Farm 4 is certified as organic farming with manure fertilization 2–3 t/dka (20–30 t/ ha), applied every 3–4 years before vegetation. Before and after flowering – several times foliar application of organic fertilizer containing macro-, micronutrients, and amino acids. Only soil tillage was carried out to control weeds. The soil tillage included 3–4 hoeing with a cultivator or manually between the rows, with biopesticides application.

Farm's	Area	GPS	Variety	Agricultural practices	Drip			
number	number coordinates			· - •				
	Со	nventional Farmi	ng (CF)					
01	Koprinka	42°38'10.74"N, 25°19'29.496"E	R. × damascena f. trigintipetala Dieck	Soil tillage, mineral fertilization, foliar feeding with NPK + microelements during vegetation, pesticides	-			
02	Damascena 1	42°40'11.6"N, 25°11'50.1"E	R. × damascena f. trigintipetala Dieck	Turf surface as mulching, mineral fertilization, foliar feeding with NPK + microelements during vegetation, pesticides	yes			
03	Damascena 2	42°40'6.60"N, 25°11'53.40"E	R. × damascena f. trigintipetala Dieck	Turf surface as mulching, mineral fertilization, foliar feeding with NPK + microelements during vegetation, pesticides	yes			
	0	rganic Farming (O	OF)					
04	Yasenovo	42°41'36.0"N, 25°16'39.8"E	R. × damascena f. trigintipetala Dieck	Soil tillage, manure application, bio pesticides	-			
05	Asen	42°38'35.8"N, 25°10'30.0"E	cv. Raduga Rosa damascena × Rosa gallica	Turf surface as mulching, manure application, bio pesticides	yes			
06	Skobelevo	42°40'16.5"N, 25°10'36.5"E	cv. Raduga Rosa damascena × Rosa gallica	Turf surface as mulching, manure application, bio pesticides	yes			

Table 1. Farming system, geographical data, variety with general agricultural practices in the studied farms.

Farms 5 and 6 are also certified as organic farming, with manure fertilization, foliar application of organic fertilizer containing macro-, micronutrients, and amino acids, with a turf surface as mulching between the rows and drip irrigation.

Plant material and sampling

We surveyed farms growing oil-bearing roses - *R. damascena* Mill. f. *trigintipetala* Dieck and cultivar Raduga [(*Rosa damascena* Mill. × *Rosa gallica* subsp. *Eryosyla Kell* var. *Austriaca* Br.) × *Rosa gallica* L.] (Nazarenko 1983). The harvest time for rose oil yield is early in the morning, between 6 and 11 a.m., to avoid temperature increase during the day, which negatively affects the yield and the quality of the rose oil (Chalova 2017). The samples of rose flowers were picked up in the morning (6–8 a.m.) within a day at the beginning of June 2019 and 2020. Three samples were randomly taken from each individual field, and each of them was collected from 40 different rose bushes. Similar experimental designs were presented by Vagn et al. (2000) for onion and peas and by Tuncay and Bostan (2010) for apricot under organic and conventional cultivation. Each sample of rose flowers (1000 g) was split into two parts. The first was used for distillation and essential oil production. The second sample of rose flowers was used for biochemical analysis.

Distillation and essential oil production

The essential oil content in the blossoms was determined after steam distillation in the Clevenger-type micro apparatus. The essential oil was measured to the graduated part of the apparatus in milliliters and was calculated as a percentage by volume (v/w). For higher accuracy, a relative density recalculation was made and was presented as a per-

centage by weight (w/w). After collection, the oil was treated with anhydrous Na,SO4 and stored in tightly closed vials at 4 °C till analysis. The analysis was performed on an Agilent 7820A GC System coupled with a flame ionization detector and 5977B MS detector. The protocol was made according to ISO 9842 for gas chromatographic analysis of rose oil. Two capillary columns: non-polar EconoCapTM ECTM (30 m \times 0.32 mm ID, 0.25 µm film thickness) and polar HP-20M (50 m \times 0.32 mm ID, $0.30 \mu m$ film thickness) were used. The first one was operated with an oven program from 80 °C (2.5 min held) to 320 °C at a rate of 10 °C/min; with 10 min held at the final temperature was applied. Hydrogen (99.999%) was used as a carrier gas at a constant flow rate of 20 ml/min. The split ratio was 1:10, the inlet temperature was set to 250 °C and the FID temperature was set to 300 °C. The non-polar column reveals a much richer spectrum of compounds and better presentation of paraffins, but it is not suitable for dividing the main terpene alcohols citronellol and nerol. They have very similar retention times and could not be split and calculated. For this reason the polar column was used for better separation. Due to the character of the HP-20M, the oven temperature program was the following: 65 °C for 0 min, then 2 °C/min to 220 °C for 10 min.

The GC/MS analysis was performed under all conditions, described above.

The ingredients were quantified by the area of FID peaks without any correction factor. The oil constituents were identified by their mass spectra, matching with the NIST and MS library, as well as whenever possible, the authentic substances were used.

Extracts preparation

Rose petals were subject to extraction in duplicate with 80% methanol in 1:15 solid to solvent ratio. The extraction was conducted in an ultrasonic bath (SIEL, Gabrovo, Bulgaria, 35 kHz, and 300 W) for 20 mins, at 65 °C. The combined extracts were used for further analysis.

Total phenolic contents

The total phenolic content was measured using a Folin-Ciocalteu reagent with slight modification (Ivanov et al. 2014).

The total flavonoids content

The total flavonoids content was analyzed using Al(NO₃)₃ reagent (Kivrak et al. 2009).

2,2-diphenyl-I-picrylhydrazyl (DPPH) assay

The rose petal water extract (0.15 mL) was mixed with 2.85 mL 0.1 mM methanol solution DPPH. After 15 min at 37 °C, the reduction of absorbance was measured at 517 nm against methanol used as a blank sample (Ivanov et al. 2014).

2,2'-azino-bi's-3-ethylbenzthiazoline-6-sulphonic acid (ABTS) assay

The rose petal extract (0.15 mL) was mixed with 2.85 ml of the ABTS solution. After 15 min at 37 °C in darkness, the absorbance was measured at 734 nm (Ivanov et al. 2014).

Ferric reducing antioxidant power (FRAP) assay

The assay was performed according to Benzie and Strain (1996).

Cupric reducing antioxidant capacity (CuPRAC) assay

The rose petal extract (0.1 mL) was mixed with 1 mL CuCl2.2H2O, 1 mL Neocuproine (7.5 mL in methanol), 1 mL 0.1 M ammonium acetate buffer and 1 mL distilled water. After 20 min at 50 °C, the absorbance was measured at 450 nm (Ivanov et al. 2014). All results for the antioxidant activity were expressed as mMTE/g.

Statistical analysis

The data were expressed as a mean \pm standard deviation (SD) from three replicates for each sample. All the results from the determination of antioxidant activity were performed in triplicates and expressed as mM Trolox equivalents (mM TE) by dry weight. To establish the influence of the agricultural practices in the studied farms on the essential rose oil composition and antioxidant activity, ANOVA statistical analysis was performed. The significant differences were tested and the P values < 0.05 were considered statistically significant. The impact of the factors was evaluated via the Coefficients of determination R². The IBM SPSS Statistics 26.0, Copyright 1989, 2019 statistical package was used to process the data.

Results and discussion

Essential oil composition

The climate data for the 2019–2020 period showed that the temperatures were much higher than the average rates for the winter season (especially 2019). This is the dormancy period for the plants. During the spring months, the temperatures were normal and higher in June, particularly in 2019. Regarding precipitation, it is obvious that in both years they were below normal. The second year of the study is characterized as wetter with more rainfall than in 2019, especially during the spring.

The chemical composition of the essential oil of rose flowers under conventionally and organically grown roses is presented in Table 2. According to the averaged data for the two periods, the productiveness of essential oil, % obtained in conventional farming (CF) was 0.04% higher than the organic system (OF) - 0.03%, with insignificant

Type of farming	Conventional farming - CF				Organic farming - OF				
Biochemical component	min	max	average	SD	min	max	average	SD	R ²
Rose oil,%	0.03	0.05	0.04 ^{ns}	0.01	0.02	0.05	0.03 ns	0.01	0.229
Ethanol	0.00	0.92	0.19 ^{ns}	0.36	0.01	0.92	0.21 ns	0.31	0.025
Linalool	0.38	0.71	0.54 ª	0.11	0.52	0.93	0.61ª	0.22	0.569
cis –Rose oxide	0.15	0.27	0.22 ª	0.05	0.00	0.27	0.16ª	0.10	0.521
trans –Rose oxide	0.08	0.14	0.12 ^a	0.02	0.00	0.14	0.08 ª	0.05	0.532
Phenylethyl alcohol	0.33	1.33	0.83 ^{ns}	0.37	0.34	1.33	0.80 ns	0.34	0.000
β-Citronellol and Nerol	25.48	33.60	29.47 ª	3.18	17.86	33.60	25.08ª	7.91	0.450
Geraniol	23.11	26.13	24.33ª	1.18	20.91	35.81	25.42ª	8.03	0.427
Eugenol	0.38	1.35	0.70 ^{ns}	0.35	0.00	1.35	0.56 ns	0.46	0.185
Methyleugenol	0.46	0.72	0.60 ^{ns}	0.10	0.00	1.32	0.50 ns	0.36	0.055
Heptadecane (C ₁₇)	0.85	2.47	1.96 ns	0.60	0.98	2.69	1.83 ns	0.68	0.000
Farnesol	0.19	3.37	1.51 ns	1.43	0.32	3.37	1.59 ns	1.32	0.003
Nonadecene ($C_{19:1}$) + Nonadecane (C_{19})	14.37	18.62	17.29ª	1.65	11.36	18.62	15.09ª	4.30	0.367
Eicosane (C ₂₀)	0.98	1.76	1.33 ns	0.27	0.52	1.77	1.11 ns	0.46	0.261
Heneicosane (C ₂₁)	4.56	8.56	6.00 ^{ns}	1.37	2.71	10.14	5.43 ns	2.26	0.053
Tricosane (C ₂₃)	0.90	2.06	1.36 ns	0.43	1.06	3.01	1.50 ns	0.70	0.139
Pentacosane (C ₂₅)	0.27	0.80	0.50 ns	0.19	0.36	1.16	0.56 ns	0.28	0.118
Heptacosane (C_{27})	0.16	0.64	0.38 ns	0.16	0.19	0.92	0.38 ns	0.21	0.013

Table 2. Biochemical composition of the essential oil of rose flowers under conventionally and organically grown roses for the two years period of study.

a,a Same superscripts within the same row represent significant differences at the level of significance P < 0.05; ns – not significant differences (P > 0.05); R2 – Coefficients of determination based on observed means.

difference. Statistically significant differences were found between the average values of the main components of essential oil composition such as geraniol, β -citronellol + nerol, linalool, cis-rose oxide, trans-rose oxide, and nonadecene $(C_{19:1})$ + nonadecane (C_{19}) . The content of geraniol, β -citronellol and neroll varied within the following range: geraniol (23.11–26.13%), β-citronellol and neroll (25.48–33.60%) for CF and (20.91–35.81%), (17.86–33.60%) for OF. (Table 2). The hydrocarbons are presented by aliphatic alkanes and alkenes. The major of them is nonadecane $(C_{19,1}) + (C_{19})$ with values between (14.37– 18.62%) for CF and (11.36–18.62%) for OF, the next one is heneicosane ($C_{\gamma_1}H_{_{44}}$), varied from (4.56% to 8.56%) for CF, and (2.71 to 10.14%) for OF. The other saturated hydrocarbons (tricosane, pentacosane, and heptacosane) do not show a certain trend and move relatively within an equal range. The phenylpropane compounds are represented by phenylethyl alcohol, eugenol and methyleugenol. The first one is most abundant in a native flower odor, but in the essential oil, its quantity is minor (Dobreva 2011; Rusanov et al. 2011; Erbaş and Baydar 2016). Due to its high water solubility, this substance is carried off with the distillation waters. It is limited in the international standard with less than 3.5%. In our study, it varies within the narrow range from 0.33 to 1.33% for CF and 0.34 to 1.33% for OF. The methyleugenol is an odor contributor, but it is not desired above a certain concentration in the essential oils due to potential cancer and allergic effects on human health (Johnson et al. 2000). The rose oil contains methyleugenol in percentages up to 5.0%, especially if the rose flowers are fermented before processing. This compound is limited and subject to monitoring. For the two-year period, the levels are relatively low: from (0.46 to 0.72%) for CF and from (0.00-1.32%) for OF. The

results of the statistical analysis showed an influence on the essential oil composition in our study case. The coefficients of determination (R^2) vary within the range from 0.367 to 0.569, thus, the influence of the type of rose oil cultivation varied from 36.7 to 56.9%. A significant difference was not found for the other components of the biochemical composition of the essential oil, and the coefficients of determination (R^2) varied within very low limits. On the basis of the results obtained, the organic farming produced essential oil with higher linalool and geraniol content and lower β -citronellol and nerol concentrations, whereas the essential oil of CF is characterized by higher β -citronellol and nerol concentrations and lower linalool and geraniol content. Similar results were obtained by Kumar et al (2017), the authors reported more flower yield plant⁻¹, number of flowers plant,⁻¹ and flower yield ha⁻¹ and a higher percentage of citronellol+nerol with an application of 120:40:90 kg NPK ha⁻¹. The authors discussed that the ratio citronellol+nerol/ geraniol is higher for fertilized plots in comparison with manure plots. In our study the ratio citronellol+nerol/geraniol was also bigger 1.21 for CF than OF - 0,98.

Antioxidant activity of the rose flower

The total phenols, total flavonoids, and antioxidant activity in methanol extracts from conventionally and organically grown roses are presented in Table 3.

The phenolic compounds, even if not directly related to the food nutritional quality, have been receiving increasing attention as a result of their specific biological activ-

Compound, %	Region	Year	Total	Total flavo-	Antioxidant activity, mM TE/g					
-	-		phenols, mg	noids, mg	DPPH	ABTS	FRAP	CUPRAC		
			GAE/g dw	QE/g dw						
Conventional	Koprinka	2019	49.01±0.22	11.07±0.23	524.32±39.89	522.74±65.06	3235.01±27.85	1713.83±147.19		
farming	Koprinka	2020	40.60 ± 13.30	9.38±1.12	596.61±128.1	544.50±111.18	955.50±333.31	456.79±52.22		
	Damas 1	2019	41.14±0.23	11.49 ± 1.44	509.57±34.58	571.33±96.56	2309.14 ± 227.49	1128.14±77.45		
	Damas 1	2020	39.50±7.3	11.13±1.30	639.64 ± 127.62	578.43±77.21	1602.74±316.61	723.13±193.31		
	Damas 2	2019	41.74 ± 0.07	11.59±0.64	476.75±41.83	607.67±79.99	2744.97 ± 189.04	1176.25 ± 145.31		
	Damas 2	2020	23.1±3.2	7.46±0.56	315.143±89.74	306.99±82.78	1062.2 ± 326.452	306.76±44.27		
	min		23.10	7.46	315.14	306.99	955.50	306.76		
	max		49.01	11.59	639.64	607.67	3235.01	1713.83		
	average		39.18 ns	10.35 ns	510.34 °	521.94 °	1984.93 ns	917.48 ns		
	SD		8.58	1.63	112.77	109.26	927.65	523.16		
Organic farming	Yasenovo	2019	63.45±0.05	12.36±0.50	1033.06±30.57	793.72±63.72	3141.96±29.02	2687.07±121.83		
	Yasenovo	2020	36.30±4.9	8.89 ± 2.27	592.86±64.63	604.33±78.95	1418.70 ± 362.67	537.34±107.42		
	Asen	2019	73.23±0.99	11.32±0.68	839.96±12.47	1033.31 ± 84.04	746.41±90.16	$2658.17 {\pm} 242.94$		
	Asen	2020	41.8 ± 12.8	13.02±2.46	675.69±179.13	676.23 ± 178.20	1301.9±609.73	687.46±169.86		
	Skobelevo	2019	69.83±4.58	11.55±1.0	754.32±29.86	1026.13±21.60	721.49±44.06	1760.29 ± 112.99		
	Skobelevo	2020	41.3±3.3	13.03±1.56	945.04 ± 326.25	652.21±45.17	3648.3 ± 1951.29	876.06±107.71		
	min		36.30	8.89	592.86	604.33	721.49	537.34		
	max		73.23	13.03	1033.06	1033.31	3648.30	2687.07		
	average		54.32 ns	11.70 ^{ns}	806.82 ª	797.66 ª	1829.79 ns	1534.40 ns		
	SD		16.32	1.55	165.60	190.27	1255.27	978.51		
	R ²		0.288	0.176	0.568	0.486	0.006	0.156		

Table 3. The total phenols, total flavonoids and antioxidant activity in methanol extracts from conventionally and organically grown roses.

a,a Same superscripts within the same column represent significant differences at the level of significance P < 0.05; ns – not significant differences (P > 0.05); R2 – Coefficients of determination based on observed means.

ity. Higher values of the total phenols were found in the methanol extract of rose petals from OF between (36.30–73.23) mg GAE/g dw, with a mean value of 54.32 mg GAE/g dw and a lower concentration for CF between (23.10-49.01) mg GAE/g dw with a mean value of 39.18 mg GAE/g. The values of total flavonoids varied between 8.89 and 13.03 mg QE/g dw with a mean value of 11.70 mg QE/g dw for OF and (7.46–11.59) QE/g dw with a mean value of 10.35 QE/g dw for CF. An interaction was found between the impact of the agricultural system and annual conditions in the study period on the total phenol and total flavonoids content, but a statistically significant difference was not found with regards to the agricultural system. The antioxidant activity of the rose petal extracts was evaluated by four methods based on the different mechanisms (DPPH, ABTS, FRAP, and CUPRAC). It was found that organic farming production (Table 3) demonstrated the best radical scavenging activity evaluated by the three DPPH, ABTS, and CUPRAC methods according to the averaged data for two years, namely 806.82, 797.66 and 1534.40 mM TE/g dw for OF versus 510.34, 521.94 and 917.48 mM TE/g dw for CF with high statistical significance for the DPPH and ABTS analyses. The coefficient of determination (R^2) showed influence of the agricultural system type on the oil bearing rose cultivation with more than 0.49% on antioxidant activity in oil rose flower. The authors' team of Wang et al. (2008) also reported significantly higher total phenolics, total anthocyanins, and antioxidant activity in blueberry fruit grown from organic culture than fruit from conventional farming. The investigation of Taie et al. (2010) with basil plants also indicated that organic fertilization can yield a significant increase in antioxidant activity, anti-cancer activity, phenolics, and flavonoids of the culinary herbal plant.

The impact of climate conditions in 2019 and 2020, irrigation practice and the variety types on the essential oil composition and the antioxidant activity of rose petals was also studied. The results are presented in Table 4.

According to Dobreva and Angelova (2011), the greatest influence on the biosynthesis of the essential oil is attributed to temperature, humidity, and light intensity, whereas the air humidity is associated with an increased production of the rose flowers and rose oil content. Therefore, the second year 2020 would be more favorable for the growth of the oil-bearing rose and the production of essential oil in the study region. As can be seen from the data presented in Table 4, the productiveness of essential oil from the studied private farms was greater in 2020 by 0.04% than it had been in 2019, without a statistical significance. Results of the statistical analysis showed that climate in the period under study was a factor influencing essential oil composition. Statistically significant differences were found between the average values of the components such as farnesol, tricosane, pentacosane and heptacosane. The coefficients of determination (R²) varied within the range from 0.35 to 0.97, thus, the influence of the climate conditions on these components varied from 35% to 97%. A statistically significant difference was also found with regards to the total phenols and the DPPH antioxidant activity, whereas the drier year of 2019 was more favorable for biosynthesis of second metabolites. This confirmed that the contents of polyphenolics and antioxidants in the rose petals (R^2 = 0.47; 0.65) are strongly influenced by the environment, e.g. the geographical and edaphic factors, as previously described by Ginova et al. (2013). Any impact of the irrigation practice on the rose oil quality and biosynthesis of second metabolites was not found. According to Ucar et al (2017) irrigation, as an agronomic practice has a significant effect mainly on the oil bearing flower yield. A statistically significant difference of the essential oil composition was found between the R. *damascena* and cv. Raduga for the main components such as geraniol, β -citronellol and neroll and eugenol. The first thing that stands out is the relationship between the most abundant terpene alcohols – for Raduga the dominant is geraniol (32.36–35.81%) and lower for R. *damascena*, between (20.91–27.24%) whereas the coefficient of determination is R² = 0.89.

Biochemical component of	min	max	mean	SD	min	max	mean	SD	R ²		
rose oil/rose flower											
	2019 Year					2020 Year					
Essential oil, %	0.02	0.04	0.03 _{ns}	0.01	0.03	0.05	0.04 _{ns}	0.00	0.01		
Farnesol	0.19	3.37	0.28	0.67	2.43	3.37	2.88,	0.34	0.97		
Tricosane (C ₂₃)	1.30	3.01	2.09	0.68	0.90	1.31	1.18,	0.17	0.54		
Pentacosane (C ₂₅)	0.45	1.16	0.77	0.30	0.27	0.53	0.42	0.98	0.44		
Heptacosane (C ₂₇)	0.26	0.92	0.52	0.24	0.16	0.38	0.28	0.08	0.35		
Total phenols, mg GAE/g dw	41.14	73.23	56.40	14.25	23.10	41.80	37.10	7.13	0.47		
CUPRAC,mM TE/g	1128.14	2687.14	1853.96	686.34	306.76	876.06	597.92	204.66	0.65		
		Irrig	ation		Non irrigation						
Methyleugenol	0.00	0.67	0.31	0.27	0.67	1.32	0.92	0.30	0.56		
Heptadecane (C ₁₇)	1.76	2.69	2.22	0.29	0.85	2.36	1.40	0.68	0.47		
		R. Dar	nascena				cv. Raduga				
Linalool	0.38	0.83	0.57	0.14	0.81	0.93	0.87	0.56	0.62		
cis –Rose oxide	0.15	0.27	0.22	0.43	0.00	0.04	0.02	0.21	0.88		
trans –Rose oxide	0.08	0.14	0.12	0.23	0.00	0.03	0.01	0.02	0.87		
β-Citronellol and Nerol	25.48	33.60	29.29	3.05	17.86	20.02	18.80	0.96	0.81		
Geraniol	20.91	27.24	24.27	1.97	32.36	35.81	34.46	1.64	0.89		
Methyleugenol	0.46	1.32	0.74	0.28	0.00	0.14	0.06	0.07	0.68		
Eicosane (C ₂₀)	0.98	1.77	1.35	0.30	0.52	0.89	0.66	0.17	0.64		
ABTS, mM TE/g	306.99	793.70	566.21	133.45	652.21	1033.31	846.97	211.27	0.45		

Table 4. Impact of the climate condition in 2019 and 2020 years, irrigation practice and variety on the rose oil composition and the antioxidant activity of rose petals.

a,a Same superscripts within the same column represent significant differences at the level of significance P < 0.05; R2 – Coefficients of determination based on observed means, SD-Standard deviation.

Conclusion

The conventional or organic agricultural type of system is a question of choice for every farmer, based on the benefits and challenges in the agricultural sector. In the medical plants cultivation, including oil bearing rose production, the choice of the system and the application of agricultural practices could have an enormous effect on the quality of cosmetic rose products and food supplements. Our results show that the application of combined eco-friendly agricultural practices in organic private farms in *R. damascena* cultivation gives a better quality of the rose flowers with higher values of antioxidant activity in comparison with the conventional agricultural system.

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References

- Baydar N, Baydar H (2013) Phenolic compounds, antiradical activity and antioxidant capacity of oil-bearing rose (*Rosa damascena* Mill.) extracts. Industrial Crops and Products 41: 375–380. https://doi.org/10.1016/j.indcrop.2012.04.045
- Benzie F, Srtain J (1996) Ferric reducing the ability of plasma (FRAP) as a measure of "antioxidant power": The FRAP assay. Analytical Biochemistry 239(1): 70–76. https://doi. org/10.1006/abio.1996.0292
- Briskin DP (2000) Medicinal Plants and Phytomedicines. Linking Plant Biochemistry and Physiology to Human Health. Plant Physiology 124(2): 507–514. https://doi.org/10.1104/ pp.124.2.507
- Chalova V, Manolov I, Manolova V (2017) Challenges for commercial organic production of oil-bearing rose in Bulgaria. Biological Agriculture & Horticulture 33(3): 1–13. https:// doi.org/10.1080/01448765.2017.1315613
- Dobreva A (2011) Technological study on the composition of rose oil. Scientific Works of University of Food Technologies 58(2): 94–98.
- Dobreva A, Angelova D (2011) Correlations between basic characteristics of the essential oil of *Rosa damascena* Mill. and environmental factors. Journal of Mountain Agriculture on the Balkans 14(5): 1086–1098.
- Erbaş S, Baydar H (2016) Variation in Scent Compounds of Oil-Bearing Rose (*Rosa damascena* Mill.) Produced by Headspace Solid Phase Microextraction, Hydrodistillation and Solvent Extraction. Records of Natural Products 10(5): 555–565.
- Erdal I, Munduz H (2017) Comparing the nutritional status of organic and conventional oilbearing rose (*Rosa damascena* Mill.) Gardens in Lakes Region With Leaf and Flower Analyzes. Toprak Su Dergisi 6(2): 26–31. https://doi.org/10.21657/topraksu.339828
- Ginova A, Mihalev K, Kondakova V (2013) Antioxidant capacity of petals and leaves from different rose (*Rosa damascena* Mill.) plantations in Bulgaria. International Journal of Pure & Applied Bioscience 1(2): 38–43. http://www.ijpab.com/form/2013%20Volume%20 1,%20issue%202/IJPAB-2013-1-2-38-43.pdf
- Giuseppina P, Lima P, Vianello F (2011) Review on the main differences between organic and conventional plant-based foods. International Journal of Food Science & Technology 46(1): 1–13. https://doi.org/10.1111/j.1365-2621.2010.02436.x
- Gunes E (2005) Turkey Rose Oil Production and Marketing: A Review on Problem and Opportunities. The Journal of Applied Science 5(10): 1871–1875. https://doi.org/10.3923/jas.2005.1871.1875

- Ivanov I, Vrancheva R, Marchev A, Petkova N, Aneva I, Denev P, Georgiev G, Pavlov A (2014) Antioxidant activities and phenolic compounds in Bulgarian *Fumaria* species. International Journal of Current Microbiology and Applied Sciences 3(2): 296–306.
- Johnson J, Ryan M, Toft J, Graves S, Hejtmancik M, Cunningham M, Herbert R, Abdo K (2000) Two-year toxicity and carcinogenicity study of methyleugenol in F344/N rats and B6C3F(1) mice. Journal of Agricultural and Food Chemistry 48(8): 3620–3632. https:// doi.org/10.1021/jf000364a
- Kivrak I, Duru M, Öztürk M, Mercan N, Harmandar M, Topçu G (2009) Antioxidant, anticholinesterase and antimicrobial constituents from the essential oil and ethanol extract of *Salvia potentillifolia*. Food Chemistry 116(2): 470–479. https://doi.org/10.1016/j.foodchem.2009.02.069
- Kovacheva N, Rusanov K, Atanassov I (2010) Industrial Cultivation of Oil Bearing Rose and Rose Oil Production in Bulgaria During 21st Century, Directions and Challenges. Biotechnology, Biotechnological Equipment 24(2): 1793–1798. https://doi.org/10.2478/ V10133-010-0032-4
- Kumar R, Sharma S, Sood S, Agnihotri K (2013) Agronomic interventions for the improvement of essential oil content and composition of damask rose (*Rosa damascena* Mill.) under western Himalayas. Industrial Crops and Products 48: 171–177. https://doi.org/10.1016/j. indcrop.2013.04.020
- Kumar R, Sharma S, Sood S, Kaundal M, Agnihotri V (2017) Effect of manures and inorganic fertilizers on growth, yield, and essential oil of damask rose (*Rosa damascena* Mill.) and chemical properties of soil in western Himalayas. Journal of Plant Nutrition 40(11): 1604–1615. https://doi.org/10.1080/01904167.2016.1270315
- Mahboubi M (2016) Rosa damascena as holy ancient herb with novel applications. Journal of Traditional and Complementary Medicine 6(1): 10–16. https://doi.org/10.1016/j.jtc-me.2015.09.005
- Malik A, SuryapanI S, Ahmad J (2011) Chemical vs organic cultivation of medicinal and aromatic plants: the choice is clear. International Journal of Medicinal and Aromatic Plants 1(1): 5–13.
- Nazarenko LG (1983) Raduga new sort essential oil rose. Scientific Works of VNIEMK. Simpheropol, Ukraine 15: 49–54.
- Nunes H, Miguel M (2017) *Rosa damascena* essential oils: a brief review about chemical composition and biological properties. Trends in phytochemical research 1: 111–128.
- Petkova N, Todorova M, Grozeva N, Gerdzhikova M (2020) Phenolic content and antioxidant activity of water extracts from *Rosa damascena* petals grown in Kazanlak Valley, Bulgaria. Scientific Papers. Series B, Horticulture. Vol. Lxiv, No. 1, 2020 Print Issn 2285–5653, Cd-Rom Issn 2285–5661, Online Issn 2286–1580, Issn-L 2285–5653. Physiology to human health. Plant Physiology 124(2): 507–514.
- Rusanov K, Kovacheva N, Atanassov I (2011) Comparative GC/MS analysis of rose flower and distilled oil volatiles of the oil bearing rose *Rosa damascena*. Biotechnology, Biotechnological Equipment 25(1): 2210–2216. https://doi.org/10.5504/BBEQ.2011.0015
- Shishkov T, Kolev N (2014) The Soils of Bulgaria. Springer Science+Business Media Dordrecht, [xvi +] 208 pp. https://link.springer.com/book/10.1007/978-94-007-7784-2

- Sobotkova A, Ross S (2018) Kazanlak valley: topography and environment in The Tundzha Regional Archaeology Project. Oxbow Books, Oxford, 38–44.
- Taie H, Salama Z, Radwan S (2010) Potential activity of basil plants as source of antioxidants and anticancer agents as affected by organic and bio-organic fertilization. Notulae Botanicae Horti Agrobotanici Cluj-Napoca 38(1): 119–127. https://doi.org/10.15835/ nbha3813534
- Todorova M, Grozeva N, Gerdzhikova M, Dobreva A, Terzieva S (2020) Productivity of oilbearing roses under organic and conventional systems. Scientific Papers. Series A. Agronomy 63(1): 580–585.
- Tuncay K, Bostan S (2010) Changes of contents of polyphenols and vitamin a of organic and conventional fresh and dried apricot cultivars (*Prunus armeniaca* L.). World Journal of Agricultural Sciences 6(2): 120–126.
- Ucar Y, Kazaz S, Eraslan E, Baydar H (2017) Effects of different irrigation water and nitrogen levels on the water use, rose flower yield and oil yield of *Rosa damascena*. Agricultural Water Management 182: 94–102. https://doi.org/10.1016/j.agwat.2016.12.004
- Vagn G, Bechmann I, Behrens A, Sturup S (2000) Comparative investigation of concentrations of major and trace elements in organic and conventional danish agricultural crops. 1. Onions (*Allium cepa* Hysam) and peas (*Pisum sativum* Ping Pong). Journal of Agricultural and Food Chemistry 48(12): 6094–6102. https://doi.org/10.1021/jf0009652
- Wang SY, Chen C, Sciarappa W, Wang CY, Camp MJ (2008) Fruit quality, antioxidant aapacity, and flavonoid content of organically and conventionally grown blueberries. Journal of Agricultural and Food Chemistry 56(14): 5788–5794. https://doi.org/10.1021/jf703775r