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Changes of scent components with animal manure and chemical fertilizer applications on *Rosa damascena* Mill.

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Abstract

This study was conducted to examine the effect of animal manure and inorganic fertilizers on the scent composition of *Rosa damascena* Mill. in 2022. Animal manure (AM), nitrogen phosphate (NP 20-20-0), diammonium phosphate (DAP 18-46), monoammonium phosphate (MAP 24-61), and triple superphosphate (TSP) were used as fertilizers, along with a control group. Headspace solid-phase microextraction gas chromatography–mass spectrometry was utilized to analyze the volatile components of roses. Major components were heneicosane (15.89–27.76%), citronellol (8.90–18.30%), nonadecene (7.90–13.83%), geraniol (6.14–8.27%), nerol (3.03–6.90%), eicosane (1.13–7.85%), and germacrene-D (2.41–5.45%). The highest percentages of citronellol (18.3%), nerol (6.9%) and total terpenes (55.77%) were obtained by animal fertilization, while the highest percentage of long–chain (aliphatic) hydrocarbon (43.60%) was obtained by NP (20-20) fertilization. The control group had the greatest level of geraniol (8.27%), followed by applications of TSP and animal manure (7.39% and 7.20%, respectively). Methyl eugenol (ME) was most abundantly produced by TSP (0.85%), whereas it was least abundantly produced by the control group (0.13%). The closest ME (0.20%) to the control group was produced by animal manure application. Based on the findings, it can be concluded that animal fertilization used in organic agriculture enhances the quality of plant essential oils.

Keywords: scent composition; fertilizer; major components; *Rosa damascena*; SPME-GC/MS

INTRODUCTION

The *Rosa damascena* Mill., plant, which yields an essential oil, is a crop of significant industrial importance cultivated across various global regions due to its valuable essential oil. The Rosaceae family's most common member is commonly referred to as the pink rose, oil rose, Damask rose, Isparta rose, Kazanlak rose, Taif rose, and Muhammedi rose. The species is cultivated in various countries including Turkey, Bulgaria, India, Iran, China, and Libya for a range of applications such as perfume, cosmetics, pharmaceuticals, food, and medicinal purposes. The two nations with the highest output of rose essential oil are Turkey and Bulgaria [1, 2]. Rosa is a genus that contains 200 species, and *R. damascena* is known to produce the highest–quality essential oil, which is widely utilized in the flavoring and fragrance industries [3-5].

The quality of *R. damascena* oil is primarily influenced by multiple variables such as genotype, pruning, fertilization, date and time of flower harvesting, harvesting stage, distillation methods, region, and agronomic factors [6-8].

Rose oil is the most expensive essential oil on the global market compared to other essential oils due to the low oil concentration in flowers and the unavailability of synthetic replacements [9]. The relative quantities of the main ingredients are the most important factors in determining the oil's quality.

A high concentration of monoterpene alcohols, such as citronellol, nerol, geraniol, linalool, and phenyl ethyl alcohol, make rose oil one of the most expensive basic materials used in the flavor and fragrance industries. The value of rose oil as a scent is mostly derived from these elements [4, 10]. The primary constituents for oil of *R. damascena* are 20–34% citronellol, 15–22% geraniol, and 8–15% nonadecane, according to the international standard for rose essential oil [6, 11-13].

Pal asserted that integrated agriculture with organic and inorganic fertilization is required to meet increasing international market demands and achieve sustainable agriculture in larger areas [14]. But unfortunately, the widespread use of nitrogen, phosphorous, and magnesium fertilizers as well as soil, air, and water pollution are significant problems in modern agriculture [15]. Organic agriculture is a different type of farming that uses natural methods without the use of chemicals. This cultivation method is intended to promote respect for the food production system's biological cycles in order to maintain and improve soil fertility, reduce pollution, avoid the use of synthetic fertilizers and pesticides, preserve genetic diversity, consider the extensive social and ecological effects of the food production system, and produce enough high–quality food [16]. Organic essential oils are more difficult to produce and more expensive than traditional essential oils, but they are an important consideration for living a healthier and longer life. When evaluating the economics of oil rose production, Ikiz and Demircan (2013) discovered that conventional production was more economical than organic production. Organic fertilization allows for the reduction of chemical residues in rose oil as well as the quantities of unwanted components (such as Methyl eugenol) that may have some negative effects due to traditional planting [17, 18].

In terms of oil rose cultivation, Mardin Province is a new territory compared to the Isparta Lakes Region, and there are positive improvements in the expansion of oil rose growing areas. Because agriculture has not been practiced in this area for many years, no chemical pesticides or fertilizers have been used. Due to the importance of improving the quality of such a valuable essential oil and since rose oil obtained without synthetic fertilizers will benefit health and be cost-effective, this study conducted in Mardin province examined the impact of animal manure and various chemical fertilizers on the production of essential oils from oil roses.

Material and methods

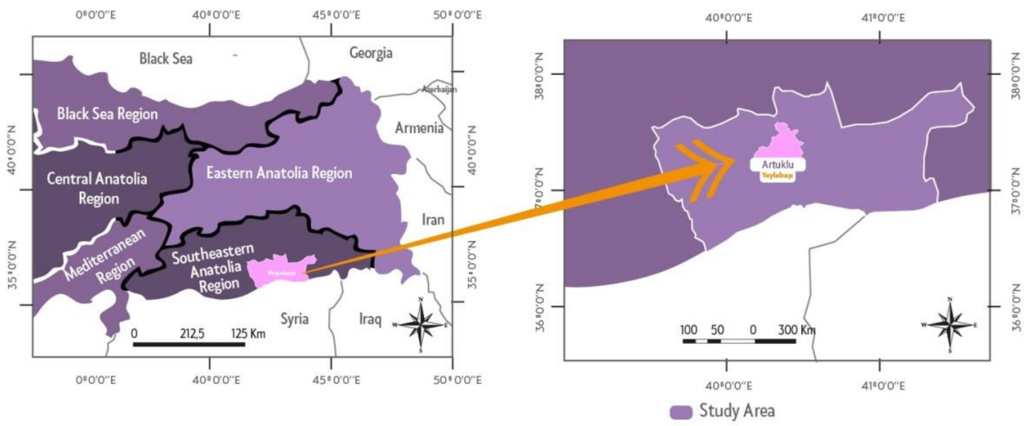

Figure 1. The experimental area for the oil-bearing rose (*Rosa damascena*) garden is depicted on this map. The trial site is a farmer's rose garden in the rural Yaylabaşı district of the Artuklu District of the Mardin Province in the Southeast Anatolia region of Turkey. This region is the second-largest producer of oil-bearing roses in Turkey, behind the Lakes Region. In addition, this region has only produced organic rose oil for the past five years.

Experimental site and soil properties

The current study was carried out at the experimental garden in the village of Yaylabaşı, which is located at 37°24'41"N 40°47'9"E and 1120 meters above sea level in the Southeastern Anatolia Region, Mardin Province. Oil rose cultivation in this new area has been going on for an average of five years on 60 hectares of farmer's land [17]. From this area, six soil samples were collected, with layers ranging from 0 to 60 cm, and delivered to laboratory for analysis of soil texture and mineral content. The soil's organic matter content (1.68%) was low and it had a loam texture. Nitrogen content was high (0.19–0.33%), copper (1.02 mg kg^{-1}), calcium (1345 mg kg^{-1}), magnesium (220 mg kg^{-1}), phosphorus (87.0 kg ha^{-1}), potassium (536.1 kg ha^{-1}), potassium (537.0 kg ha^{-1}), manganese (5.44 mg kg^{-1}), and zinc (0.52 mg kg^{-1}) levels were low, limy (2.79%), slightly alkaline (7.5–8.5%), and salt–free (0.02%) (Figure 1).

Experimental design and treatments details

Each fertilizer experimental plot had three rows and total eighteen plants, with a 2 m distance between inter–rows and a 4 m distance between intra–rows. The experiment consisted of six treatments, viz., control group, animal manure (AM), nitrogen phosphate (NP 20-20-0), diammonium phosphate (DAP 18-46), monoammonium phosphate (MAP 24-61), and triple superphosphate (TSP) fertilizers. The NP fertilizer was mixed into the soil in band form, in line with the plant trace. DAP fertilizer was applied to the plant trace and at a depth of 10–15 cm of soil in mid-March. Starting from March, MAP fertilizer was applied with drip irrigation method 5 times at 15-day intervals. TSP fertilizer was mixed into the soil before flowering and applied in the form of bands. Animal manure was applied by mixing with the soil in the autumn.

Sample preparation

The flower harvest was done on May 27, 2022. The samples were transported to the laboratory in a thermos set at +4 °C. Rose samples were collected for analysis in the May 27. Each time, the flowers were collected around 6:00 a.m. Samples for SPME-GC/MS analysis were made by mixing flowers collected in triplicate from various rose bushes. The oil quality (the ratio of terpene alcohols) in rose flowers harvested at this hour is high due to the high humidity and low temperature (Table 1) [11]. Flowers were collected in three replications from separate plant bushes for each application. For each application, flowers from various rose bushes were mixed together to create samples that were selected randomly. Oil rose flowers was homogenized with a crush and then used to identify volatile components. This method facilitates the detection of fragrance components.

Table 1. Harvest day and harvest time average temperature and relative humidity values*

Day/Hour	Average temperature (°C)	Relative humidity (%)
May 27	21.1	30.1
06:00	13.0	47.0

*Meteorological data were obtained from the Mardin Meteorology Directorate.

Solid Phase Micro-Extraction-Gas chromatography/Mass spectrometry (SPME-GC/MS) analysis conditions of flower samples

Using the headspace HS-SPME procedure, The Supelco DVB /CAR/ PDMS fiber was utilized for the measurement. The scent components were analyzed using a SHIMADZU GC/MS-QP2020 (Gas Chromatography-Mass Spectrometry) analyzer system with FID and MS detector. A DB WAX capillary column (Rtx-2330 RESTEK 60 m×0.25 mm×0.2 µm) was used in the system. The injection split ratio mode was set to 25:1, and the system pressure was set to 80 kPa. As a carrier gas, helium 5.0 (purity 99.999%) was used. The temperature of the injector block was 240°C. The oven program was 40°C for 2 minutes, then 4°C/min to a final temperature of 240°C with a 3 minute holding period. The injection volume was 1 µL and the FID temperature was 250°C. The components were identified by comparing their linear retention indices and mass spectra to those of reference standards [19].

Results

Scent composition

Table 2 illustrates the scent composition of oil rose flowers based on the different fertilizer applications applied. As a result of the SPME-GC/MS analysis, 35 components were identified. In sequence of fertilizer application, the total essential oil ratios were 97.26%, 95.45%, 97.73%, 95.46%, 95.49%, and 97.59%. These included eight monoterpenes, five oxygenated monoterpenes, seven sesquiterpenes, one oxygenated monoterpene, six long chain hydrocarbons, and seven other components.

Major components

The major components of essential oil were: heneicosane (15.89–27.76%), citronellol (8.90–18.30%), nerol (3.03–6.90%), nonadecene (7.90–13.83%), geraniol (6.14–8.27%), eicosane (1.13–7.85%), and germacrene-D (2.41–5.45%). The component contents of rose flowers, particularly the major components, varied due to different fertilizer treatments (Table 2).

Terpenes

Eight monoterpenes, six oxy monoterpenes, seven sesquiterpenes, and one oxy sesquiterpene constitute the terpenes in this study. In six distinct applications, the overall terpene ratios were 55.34%, 55.77%, 50.84%, 49.50%, 53.69%, and 52.14%, respectively. The application of animal manure produced the greatest terpene ratio, at a rate of 55.77%. The application of MAP fertilizer yielded the highest total monoterpene concentration (17.5%), while animal manure application yielded the lowest value (11.04%). The application of DAP (18-46) yielded the highest concentration of sesquiterpene (14.89%), whereas the application of TSP yielded the lowest concentration (7.34%) (Figure 2). The control group got the lowest value of farnesol (1.22%), while DAP (18-46) fertilizer application yielded the largest amount (1.99%). The essential oil with the highest concentration (34.98%) of total oxygenated monoterpenes, which is what gives rose oil its distinctive scent, was produced by applying animal manure, whereas the lowest concentration (19.20%) was produced by DAP (18-46) fertilization (Table 2).

Long–chain (aliphatic) hydrocarbons

The total of long–chain hydrocarbons (36.73–43.60%), as shown in the table, had the largest proportion of scent components in all applications. The application of NP (20-20) fertilizer produced the greatest amount of long-chain hydrocarbons (Table 2).

Methyl eugenol (ME)

The control group had the least value of methyl eugenol (0.13%), whereas the TSP fertilizer treatment had the greatest concentration (0.85%) (Table 2).

Discussion

In this research, it was determined that different fertilizer applications caused considerable changes in the scent components of the oil-bearing rose. This conclusion was consistent with the findings of previous research that show that various fertilizers and fertilizer treatments affect essential oil components [9, 13, 18]. Given that the total terpene compound content was highest and the sum of long-chain hydrocarbons was lower than the other fertilizations, we can conclude that animal fertilization improves essential oil quality.

The major components of rose essential oil were found to be citronellol, nerol, Linalool, *β*–Myrcene, geraniol, germacrene-D, Nonadecane, eicosane, and Heneicosan in a study with various fertilization and hormone applications. Furthermore, the major components were obtained in the greatest quantity from calcium nitrate $Ca(NO_3)_2$ fertilization [20]. It has been shown that different fertilization studies on *R. damascena* provided different levels of heneicosane (2.87–15.73%), citronellol (8.90–18.30%), nerol (3.03–6.90%), nonadecane (2.65–23.4%), geraniol (5.55–35.6%), eicosane (0.1–1.48%), and germacrene-D (0.57–3.17%) components [18, 20, 21]. The following factors influence the different amounts of these components: fertilizer type, amount, and application method; a different environment and climate. Example, Izgi has identified linalool, citronellol, nerol, geraniol, and nonadecane as the major components of essential oils of roses collected on different dates [17]. In this study, the application of animal manure had the highest total citronellol and nerol (Table 2). The total ratio (C+N) of these two components reached ISO (International Organization for Standardization) norms (25.1–30.1%) [22]. The total levels of citronellol and nerol obtained in the other five applications, on the other hand, were below ISO standards. Kiymaz et al. found nine varied outcomes in their investigation of different water regimes and nitrogen fertilizations, with citronellol + nerol ratios ranging from 21.15% to 42.67% [21]. The C+N ratio has been reported to be 25.08% for organic production and 29.47% for conventional production in another study [18]. The C + N ratios in a study that examined different harvest periods ranged between 26.13% and 51.34% [17]. According to another study that looked at the effects of foliar fertilization with $Ca(NO_3)_2$ and seasonal periods, C + N ratios ranged from 34.32% to 36.92% [23].

The two most significant monoterpenes, citronellol and nerol, were determined to be considerably elevated by animal manure in this study. Additionally, DAP fertilization provided the closest application (1.45%) to the desired citronellol/geraniol (C/G) ratio range (1.25–130%) [4] for the ideal odor of rose oil. Ali et al. have reached the optimal ratio range in six separate pruning and phosphorus (P) fertilizer treatments, but only achieved it in one [24]. In another study, potassium and zinc applications to the leaves of the oil rose plant increased essential oil yield, and the optimal C/G ratio has been obtained (1.25–1.26%) in all seven applications [25]. Citronellol and geraniol are monoterpenes that are important in determining the essential oil quality of rose oil. Antifungal and good growth inhibitors include monoterpenes like citronellal, citronellol, nerol and geraniol [26, 27]. They also have protective effects against food mites [28].

In numerous research, citronellol and nerol, both monoterpenes, have been written as citronellol + nerol. The argument given is that it is impossible to discern between them (or similar ones) since their retention times are so close to each other. This problem is assumed to be connected to the technical parameters of the column in the GC/MS apparatus (such as column length and diameter). Terpenoids generally have antibacterial, antifungal, antiviral, antihyperglycemic, anti-inflammatory, and antiparasitic effects and have been shown to be useful in chemotherapy and chemoprevention [29]. It is preferable to raise the overall contains of terpenes naturally in plant essential oil. The oil-bearing rose flowers with the highest total terpene concentration were taken at the early harvest date, according to a study on several harvest dates (Izgi 2022).

All rose flowers included volatile compounds, although aliphatic (long–chain) hydrocarbons were the most major form [30]. Essential oil components are dynamic, and a wide range of factors, including climate, timing and length of the harvest, fertilizer, irrigation, and pruning, can change both their composition and quantity. The essential oil of *R. damascena* grown in the Himalayas contained around 21.23% (aliphatic) long-chain hydrocarbons [31]. In a separate study involving 12 flower color mutation variants of *R. hybrida*, the scientists discovered that the essential concentrations of aliphatic hydrocarbons ranged from 63.1% to 80.9% in all genotypes of roses [30]. It is preferable

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for long-chain (aliphatic) hydrocarbons to continue to exist in rose essential oil in a manageable quantity since they offer permanence in the fragrance.

It has been revealed that methyl eugenol is found in all 450 plants from 80 families and that these plants employ it to defend themselves against herbivores, pathogenic microorganisms, insects, and some neighboring plants [32-34]. ME contributes to odor, but it has been recommended to avoid or keep it to a minimum as rose oil is a carcinogenic, mutagenic, and allergenic ingredient [35]. The ME concentration of rose flowers obtained by Rusanov et al. at seven distinct floral stages was quantified and analyzed independently. They discovered that the third and fourth stages of flowers, which are in bud form, contain the least amount of ME [36]. The ME content of rose essential oil must be kept at a safe level that will not impair human health. As a result, more research is needed to discover what circumstances cause the ME content of essential oils to drop or increase. In a nutshell, each element affects the components of the oil-bearing rose's essential oils.

Table 2. Animal manure and various chemical fertilizers' scent components change in *R. damascena*

					Fertilizer type					
	RT	RI[a]	RI[b]	Components	Control	AM	NP	DAP	MAP	TSP
				Monoterpenes	Content (%)					
1	11.460	1120	1035[1]	*α*-Pinene	7.56	6.71	9.82	7.85	8.93	9.17
2	14.850	1203	1118[1]	*β*-Pinene	2.33	0.95	1.50	1.27	2.48	1.42
3	15.555	1219	1245[1]	*β*-Phellandrene	0.82	0.85	1.19	0.92	1.92	0.97
4	17.685	1264	1158[1]	*β*-Myrcene	2.22	1.96	3.85	2.85	2.92	2.67
5	18.375	1279	1184[1]	*α*-Terpinene	0.09	0.16	-	0.08	0.18	0.10
6	19.260	1298	1208[1]	*d*-Limonene	0.13	0.11	0.27	0.16	0.46	0.18
7	21.400	1348	1249[1]	*γ*-Terpinene	0.17	0.18	0.13	0.16	0.33	0.19
8	21.690	1355	1355[3]	*β*-cis-Ocimene	0.11	0.12	-	0.13	0.28	0.11
				Total	**13.43**	**11.04**	**16.76**	**13.42**	**17.5**	**14.81**
				Oxygenated monoterpenes						
9	27.605	1507		Rosefuran	0.15	0.15	0.13	0.14	0.08	0.15
10	32.100	1648	1544[1]	Linalool	1.32	1.02	0.12	0.12	0.12	0.18
11	37.200	1843	1840[3]	Citral	1.39	1.41	0.89	0.61	0.66	0.73
12	37.885	1865	1768[1]	Citronellol	16.24	18.30	9.56	8.90	10.54	15.06
13	38.943	1902	1792[1]	Nerol	5,54	6.90	3.54	3.29	3.03	4.61

14	39.665	1947	1852[1]	Geraniol	8.27	7.20	6.73	6.14	6.23	7.39
				Total	**32.91**	**34.98**	**20.97**	**19.20**	**20.66**	**28.12**
				Sesquiterpenes						
15	31.595	1631	1624[3]	*β*-Bourbonene	0.20	0.29	0.38	0.55	0.47	0.22
16	33.705	1703	1437[2]	*α*-Guaiene	1.32	1.22	2.32	2.52	2.17	0.91
17	34.010	1714	1712[3]	Caryophyllene	1.42	1.48	2.28	2.56	3.07	1.75
18	35.890	1790	1787[3]	*α*-Humulene	1.01	1.35	1.66	1.86	1.37	0.66
19	36.880	1829	1827[3]	Germacrene-D	2.41	2.42	3.58	5.45	4.68	2.74
20	37.095	1834	1831[3]	*α*-Bulnesene	1.42	1.34	1.34	1.67	1.89	0.69
21	37.580	1855		*α*-Farnesene	-	0.19	0.32	0.28	0.32	0.37
				Total	**7.78**	**8.29**	**11.88**	**14.89**	**13.97**	**7.34**
				Oxygenated sesquiterpenes						
22	50.645	2460	2452[3]	Farnesol	**1.22**	**1.46**	**1.23**	**1.99**	**1.56**	**1.87**
				Long–chain hydrocarbons						
23	30.605	1598	1700[1]	Heptadecane	1.24	0.33	0.96	1.46	1.65	2.02
24	33.575	1698	1600[1]	Hexadecane	0.35	0.24	-	0.42	0.30	0.61
25	38.005	1899	1800[1]	Octadecane	0.25	0.18	-	0.42	0.29	0.50
26	40.910	2001	1995[3]	Heneicosane	22.60	26.03	25.95	26.88	15.89	27.76
27	41.766	2036	2014[3]	Nonadecene	9.48	7.90	13.83	12.10	10.75	8.40
28	42.695	2100	2000[1]	Eicosane	4.51	2.62	2.86	2.60	7.85	1.13
				Total	**38.43**	**37.3**	**43.6**	**43.06**	**36.73**	**40.42**
				Other components						
29	25.595	1453	1362[1]	1-Hexanol	0.14	0.15	-	0.34	0.43	0.54
30	34.225	1770	1639[1]	Citronellyl acetate	0.71	0.59	0.95	0.79	1.50	0.70
31	36.877	1821		*n*-Pentadecanol	1.24	1.01	1.14	2.01	1.00	2.05
32	39.360	1933	1785[1]	Phenethyl acetate	-	0.12	0.20	0.24	0.43	0.12
33	40.005	1985	1879[1]	Benzyl alcohol	0.13	0.09	0.13	0.07	0.12	0.24
34	43.525	2119		Methyl eugenol	0.13	0.20	0.41	0.21	0.48	0.85
35	46.480	2285	2164[1]	Eugenol	1.14	0.22	0.46	0.24	1.11	0.53
				Total	**3.49**	**2.38**	**3.29**	**3.90**	**5.07**	**5.03**
				Overall Total	**97.26**	**95.45**	**97.73**	**95.46**	**95.49**	**97.59**

RT: Retention Time, RI^a: Retention index calculated on DB WAX column, RI^b: The relative linear retention index from the literature (1: [37], 2: [38], 3: [17])

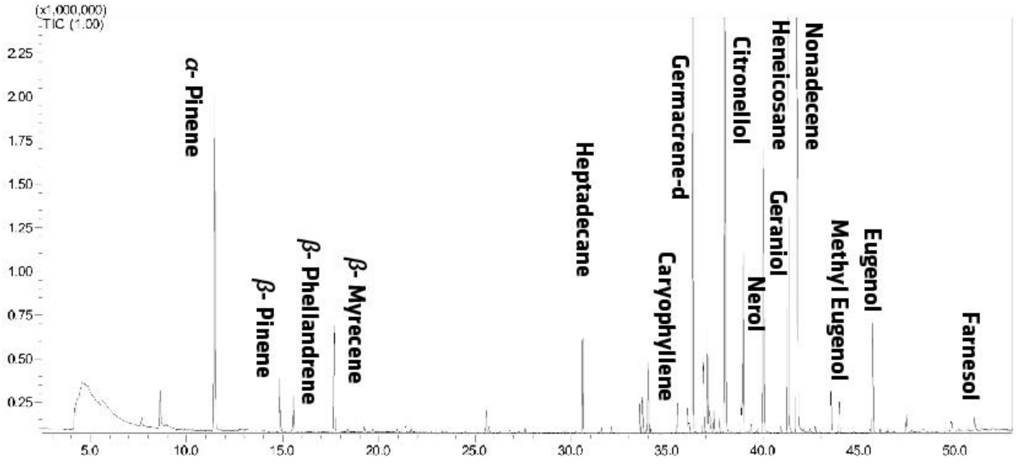

Figure 2. GC chromatogram of *R. damascena* fertilized with Triple Superphosphate (TSP)

CONCLUSION

Rosa damascena essential oil is unique among essential oils in that it is used in the food, cosmetics, pharmaceutical, and perfume industries and cannot be synthetically imitated. At the end of the study, the highest concentrations of total terpenes, and citronellol and nerol, as well as the lowest quantity of methyl eugenol among fertilizer applications, demonstrated that the use of animal manure had a substantial impact on the improvement in essential oil quality. In addition, MAP fertilizer application (36.73%) followed by animal manure application (37.30%) yielded the lowest aliphatic (long chain) hydrocarbon levels. In conclusion, animal manure has been demonstrated to be more effective than inorganic fertilizers in increasing essential oil quality in the production of oil roses. It is advised that animal manure be utilized instead of inorganic fertilizer to decrease soil-water pollution and improve essential oil quality in oil-bearing rose agriculture. A more extensive study incorporating solely different organic fertilizer treatments may be necessary.

Disclosure statement

The author reports there are no competing interests to declare.

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