ISSN 1021-4437, Russian Journal of Plant Physiology, 2023, Vol. 70:220. © Pleiades Publishing, Ltd., 2023.

RESEARCH PAPERS

Foliar Application of Chelated Zn and Fe Fertilizers Changed Secondary Metabolites and Essential Oil Production in Different Damask Rose Landraces

M. Kanani[a], E. Chamani[a], *, A. A. Shokouhian[a], and R. Nabipour Sanjbod[a]

[a] *Department of Horticultural Sciences, Faculty of Agriculture and Natural Resources, University of Mohaghegh Ardabili, Ardabil, Iran*

**e-mail: echamani@uma.ac.ir*

Received November 22, 2023; revised January 13, 2024; accepted January 16, 2024

Abstract—Today, essential oils (EOs), one of the most important natural products, have substituted pharmacologically relevant agents. *Rosa damascena* Mill, as an oil-bearing plant, has been widely used for this purpose. However, modern techniques to increase EOs, secondary metabolites (SMs), and chemical compounds of rose landraces have been less studied. Recently chelated Zinc (C-Zn) and chelated Iron (C-Fe) have affected SMs and EO compositions. Hence, a Split-split plot experiment on a randomized complete block design was conducted to investigate the foliar application of C-Zn (0, 1, 2, and 4 g L^{-1}) and C-Fe (0, 1, 2, and 4 g L^{-1}) on three landraces [Qamsar (QA), Khoy (KY), and Gounbarf (GN)]. HPLC and GC-MS analysis revealed the significant differences accrued via the foliar application of chelated fertilizers. C-Zn and C-Fe (4 g L^{-1}) produced the highest level of proline content and phenylalanine ammonia-lyase (PAL) enzyme activity. A positive correlation was found between proline content and PAL activity. Phenolic acids (gallic, caffeic, chlorogenic, and coumaric) and flavonoids (rutin, quercetin, kaempferol, and apigenin) were found in the methanolic extract of Damask rose. The most abundant compounds in QA were nonadecane (22.43%), citronellol (12.76%), geraniol (11.23%), and heneicosane (10.18%), respectively. Results indicated that genetic diversity is probably one of the factors that can affect SMs and EO production in Damask rose landraces. Furthermore, chelated fertilizers affected phenolic acids, flavonoids, and EOs composition. Overall, C-Zn and C-Fe application on QA could increase in antioxidant activity of produced compounds and act as a suitable treatment to be used in therapeutic processes.

Keywords: *Rosa damascena*, citronellol, Damask rose landraces, GDBH framework, geraniol, PAL activity

DOI: 10.1134/S1021443723603191

INTRODUCTION

Due to the health-promoting properties of natural products, there is an ever-increasing interest in researching the use of natural products in the pharmaceutical industry [1]. SMs are not required for the plant life cycle. However, SMs are vital for interaction with the environment, enhancing plant defense systems, defending against herbivores, and pharmaceutical processes. SMs can help alleviate disease symptoms, promote human health, and inhibit some disorders, such as human skin disorders and cancer [2]. EOs are a mixture of highly valuable volatiles that contribute to the fragrance and flavor of a plant. EOs have long been studied for their beneficial effects in human health, industrial, cosmetic, aromatherapy, and pharmaceutical applications [3].

Rosa damascena Mill, one of the most significant sources of oil-bearing plants, originated from Damascus and is called Damask rose. Damask rose has numerous landraces [4]. Landraces are the distinct genotypes within a species that have favored adaptation to the local environment. During restoration in the new environment, different changes in genetic background and growth conditions can happen in landraces. Although there are many landraces in Iran, the landraces of Iran have been studied to a limited extent [5].

Damask rose possesses EO with antibacterial, antidiabetic, anti-inflammatory, antioxidant, and antiviral properties, so it is widely used in the perfumery, cosmetics, and medicinal industries. Over the years, Taif's Rose and the landraces of Iran have been widely used for EO and rose water [6]. In addition, various constituents such as alkaloids, glycosides, terpenes, phenolic acids, flavonoids, and anthocyanins have been detected in the petals and hips of Damask rose.

Abbreviations: C-Zn, chelated zinc; C-Fe, chelated iron; PAL, phenylalanine ammonia-lyase; QA, Qamsar; KY, Khoy; GN, Gounbarf; SMs, secondary metabolites; EO, essential oil.

Table 1. Soil physicochemical properties

Depth, cm	EC, ms/cm	pH	SP, %	Clay, %	Silt, %	Sand, %	Tex.	T.N.V, %	OC, %	N	Pava, ppm	Kava, ppm	CEC
0–60	1.2	7.9	55	14	55	31	Silt	17.25	1.2	0.3	11.5	256	8

EC, Electrical conductivity; SP, Saturation percentage; Tex, Texture; T.N.V, Total neutralizing value; OC, Organic carbon; N, Nitrogen; P_{ava}, Available phosphorus; K_{ava}, Available potassium; CEC, Cation exchange capacity.

Also, significant amounts of cardiac glycosides, flavonoids, and phenolics have been found in the floral solid distillation wastes [7].

In the Kashan landrace, some compounds represented more than 95% of the total oil, such as β-citronellol, nonadecane, geraniol, nerol, and kaempferol [8]. Baydar and Baydar [9] reported gallic acid, syringic acid, and quercetin in the flower extract of Damask rose. Moreover, gallic acid, rutin, quercitrin, myricetin, quercetin, and kaempferol were detected via HPLC analysis in the flower extract of *Rosa damascena*. Kashan and Tabriz's landraces of produced different levels of SMs. Rasouli et al. [10] reported that four landraces of *Rosa damascena* (Ghalhar, Lavasanat, Kamoor, and Minab) produced different levels of polyphenols, phytochemicals, and scent composition during flower development. Identifying the chemical composition of rose oil has been the objective of many researchers.

Micronutrients are used in low quantities. However, they are vital for the many physiological functions of plants. Micronutrient deficiencies often occur in semi-arid regions and soil with high pH, salinity/alkalinity problems, poor structure, and low organic carbon content [11]. Zinc (Zn) is an essential micronutrient, involved in a range of plant processes, including antioxidant enzymes activity, protein synthesis and subsequently regulating gene expression, and utilization of carbon in terpenoid biosynthesis. Iron (Fe), as a micronutrient, is present in ferrous (Fe^{2+}) and ferric (Fe^{3+}) ions and is vital in the synthesis of chloroplast-protein complexes and is involved in redox reactions [12]. It has been reported that the availability of nutrients and the type of nutrient element can affect SMs production and EO composition [13]. Further, N-Fe and N-Zn modified the EO of *Eruca sativa* Mill. [11]. Foliar application of N-Zn affected the composition of EO in *Satureja hortensis* L. [14]. Moreover, foliar application of boron (B), Zn, and Fe significantly affected EO production in *Mentha piperita* L. [15]. Furthermore, the maximum oil yield was observed with Zn + Fe foliar application in *Brassica napus* [16]. In El-Sonbaty's study, foliar applications of Rose-scented geranium with N-Fe significantly increased the amount of EO [17].

Irreparable damages have occurred in the soil culture due to the large-scale application of fertilizers. Targeted delivery and slow and controlled release can effectively increase the effect of fertilizers on plant performance. Therefore, here efforts have been made to study the effect of various concentrations of C-Zn and C-Fe on SMs content and EO composition of *Rosa damascena* in different landraces to expand its utilization in cosmetics, perfumery, and therapeutic processes.

MATERIALS AND METHODS

Plant material. In order to investigate the effect of chelated fertilizers, three landraces of Damask rose (Qamsar (QA), Khoy (KY), and Gounbarf (GN) were considered. Cuttings of different landraces (15 cm) were collected from various geographical regions of Iran during the dormant season and planted in a greenhouse for rooting. After rooting, the plants were transferred to the main farm and cultivated according to the statistical design. The experiment was conducted in 2016–2019. The main farm was located in Urmia City (372532.7 N, 451202.9 E, and altitude of 1300 m), West Azerbaijan province, Iran. Urmia City has a semi-arid and cold climate with an average annual rainfall of 250 mm. Table 1 indicates some physicochemical properties of the soil (depth of 60 cm) in the experimental site.

Layout, design and intercultural operation. This experiment was conducted with a randomized complete block design arranged as a Split-split plot with three replications (each included three plants). In the present study, there were 3 experimental factors: type of chelated fertilizers (C-Zn and C-Fe), concentrations of chelated fertilizers (0, 1, 2, and 4 g L^{-1}), and landraces (QA, KY, and GN). Weeds were controlled mechanically, and plants were irrigated once a week (pH 7.8). After restoration and during the second year, the content of SMs and EO composition were evaluated.

Application of chelated fertilizers**.** In this experiment, two types of chelated fertilizers were used: C-Zn (containing 12% chelated zinc and absorbable at pH 3–11) and C-Fe (containing 9% chelated iron and absorbable at pH 3–11). Zinc and iron chelated fertilizers (with Khazra trademark) were produced by the Sodour Ahrar Shargh Company (IRAN) under a patent registered at the United States Patent and Trademark Office (USPTO) [US8288587B2. MH N. Chelate compounds. Google Patents 2012]. For treatment, different concentrations of C-Zn and C-Fe were prepared. Foliar application of fertilizers was done twice monthly, during the growing season.

Proline. Bates et al. [18] method was applied for proline measurement. Briefly, 50 mg of dried leaf tissue was homogenized in 1 mL sulfosalicylic acid 3% and then centrifuged for 10 min at 3944 *g*. Then, 2 mL of supernatant, 2 mL ninhydrin reagent (Merck; Germany), and 2 mL glacial acetic acid were mixed and transferred to the heat bath (100°C) for 1 h. The reaction was stopped using an ice-water bath. Then, two separate layers were formed by adding 8 mL toluene (Merck; Germany) to the mixture. The absorbance of the supernatant was read at 520 nm with a spectrophotometer (Jenway 6705 UV/Vis; UK). Proline content was calculated using a standard curve according to formula and expressed as μmol g^{-1} dry wt (Fig. 1).

$$\text{Proline content} = [\mu\text{g proline/mL} \times \text{mL toluene}/115.5\ \mu\text{g/mmole}]/[(\text{g sample}/5)].$$

Phenylalanine ammonia-lyase (EC: 4.3.1.5) enzyme activity. PAL activity was determined from 0.5 g of freeze-dried petals in 6.5 mL Tris-HCl buffer (50 mM, pH 8.8) containing 15 mM β-mercaptoethanol. The samples were ground finely and then centrifuged at 50000 *g* for 30 min at 4°C. The supernatant was used for the enzyme assay, based on Wang et al. [19] method according to the rate of cinnamic acid production. Briefly, 1 mL of extraction buffer, 0.5 mL of 10 mM L-phenylalanine, 0.4 mL of deionized water, and 0.1 mL of enzyme extract were mixed and incubated at 37°C for 1 h. The reaction was stopped by adding 0.5 mL of HCl 6 M, and the product was extracted in 15 mL ethyl acetate. The extracting solvent was evaporated by putting the extract at room temperature, and the solid residue was suspended in 3 mL of 0.05 M NaOH. The cinnamic acid concentration was calculated at 290 nm wavelength using a spectrophotometer (SP-UV 200, Spectrum Instruments Limited, Australia). A unit of enzyme activity was defined as the amount of PAL that produced 1 μM of cinnamic acid in 1 min.

HPLC analysis of secondary metabolites. Freeze-dried petal samples were ground with liquid nitrogen, and 500 mg was extracted with 2 mL methanol containing 3% (v/v) formic acid and 1% (w/v) 2,6-di-tert-butyl-4-ethylphenol (BHT) in an ultrasonic bath for 1 h and treated samples were centrifuged for 7 min at 15777 *g*. A chromafil AO-20/25 filter (Macherey-Nagel Düren, Germany) was used for filtering the supernatant. HPLC Agilent 1100: USA, with a diode array detector at 250, 272, and 310 nm was used to analyze the extract. A Nova pack C18 (250 × 4.6 mm; 4 μm) HPLC column at 25°C was used. The injection volume and the flow rate were 20 μL and 1 mL min^{-1}, respectively. The elution solvents were (A): aqueous 1% formic acid and (B): acetonitrile. The samples were eluted according to the linear gradient described by Marks et al. [20].

Acetic acid (Solvent A) solution and acetonitrile (Solvent B) were applied as the mobile phase involved 1% aq. The gradient elution was modified from 10 to 40% B in 28 min, 40 to 60% B in 39 min, and 60 to 90% B in 50 min. The mobile phase composition back to the initial condition (solvents B : A, 10 : 90) in 55 min and allowed to run for another 10 min. The total analysis time per sample was 15 min. Compounds were identified according to their retention time and by spiking with standards under the same conditions. The content of samples was measured by calculating the integrated peak area, and the content was calculated using the calibration curve by plotting the peak area against the concentration of the respective standard sample (Fig. 2).

GC-MS analysis of essential oils. The EOs of different landraces were prepared using a Clevenger apparatus (2000 mL) from hand-picked rose petals in the early morning [21]. The rose petals (100 g) were suspended in water (300 mL). Hydro-distillation was done for 4 h. The collected EO was dried over anhydrous sodium sulfate and stored in sealed vials, at a low temperature (4°C), before injection into the GC-MS. A GC (Agilent Technologies, Santa Clara, CA, USA) equipped with MS (Agilent Technologies) 5975C mass detector was applied for analysis. The splitless injector was operated at 250°C with a purge flow of 50 mL min^{-1} for 2 min. HP-5 MS column (Agilent Technologies) with 30 m × 0.25 mm of internal diameter and 0.25 μm film thickness was used. Helium gas at a flow rate of 1.5 mL min^{-1} was used as carrier gas. The temperature of the column was held at 80°C for 3 min, then increased at a rate of 8°C min^{-1} to 180°C and held for 3 min before increasing to a final post-run temperature of 240°C for 5 min. The HP 5975C mass detector parameters included; electron impact ionization (EI) source temperature: 230°C; interface temperature: 280°C; ionization energy: 70 eV; mass range: 40–500 amu; quadrupole temperature: 150°C. The compounds were identified using the Mass Spectral Library

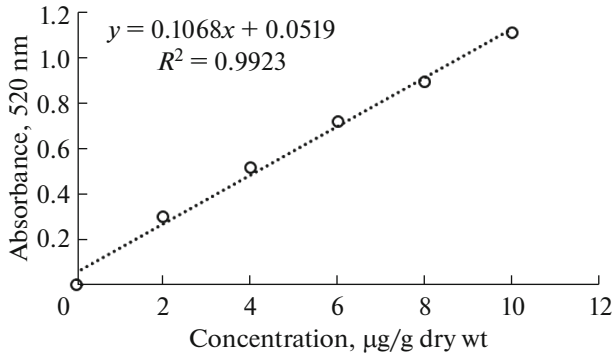

Fig. 1. Standard curve of proline for determination of proline content in Damask rose.

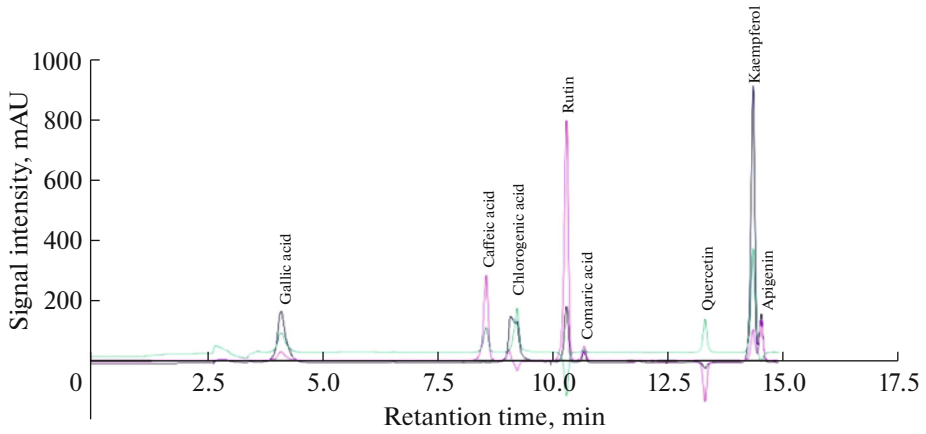

Fig. 2. Standard curve of polyphenols (gallic acid, caffeic acid, chlorogenic acid, rutin, coumaric acid, quercetin, kaempferol, apigenin) in Damask rose petals using the HPLC analysis with a diode array detector at 250 nm (green line), 272 nm (black line), and 310 nm (pink line).

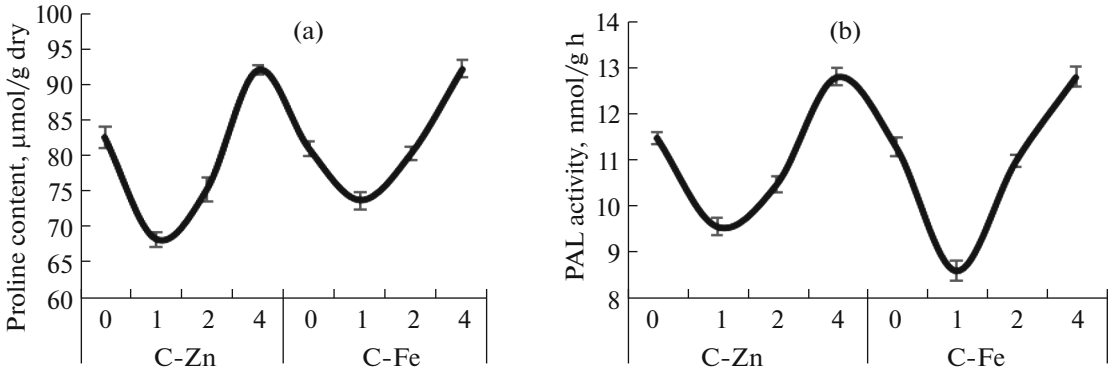

Fig. 3. Effect of chelated fertilizers concentrations on proline content (a) and PAL enzyme activity (b) in *Rosa damascena* extract.

(edition 7n.1; Wiley, Hoboken, NJ, USA). Their retention indices were compared, and the area under the curve of each compound was measured to determine their relative abundances.

Statistical analysis. Data analysis was performed using SAS V9.2 software (SAS Institute Inc., Cary, NC, USA). Means were compared using Duncan's multiple range tests at $P \leq 0.01$ and $P \leq 0.05$.

RESULTS

Proline Content

Chelated fertilizers treatments significantly affected the proline content in different *Rosa damascena* landraces. Chelated fertilizers at 1 g L^{-1} significantly reduced the proline content. The increase in chelated fertilizers concentrations increased the proline production, where the highest proline content was produced in chelated fertilizers at 4 g L^{-1} (Fig. 3a). The response of different landraces to chelated fertilizers treatments for proline production was more or less the same, hence some differences were observed. Chelated fertilizers at 1 g L^{-1} reduced the proline content in all studied landraces, although the content of proline at 1 g L^{-1} in the KY landrace was higher compared to QA and GN (Table 2). The proline content was higher in QA landrace at 2 and 4 g L^{-1} concentrations compared to KY and GN landraces (Table 2).

Phenylalanine Ammonia-Lyase Activity

PAL enzyme activity was significantly affected by landrace, chelated fertilizers concentrations, and the interaction effect of chelated fertilizers × chelated fertilizers concentrations. Chelated fertilizers at 4 g L^{-1} resulted in the highest level of PAL activity (Fig. 3b). Further, the QA landrace showed the highest PAL activity, followed by GN (Fig. 4a). Changes in proline content directly affected the PAL activity, and there was a positive correlation ($r^2 = 0.8544$) between proline content and PAL activity (Fig. 4b).

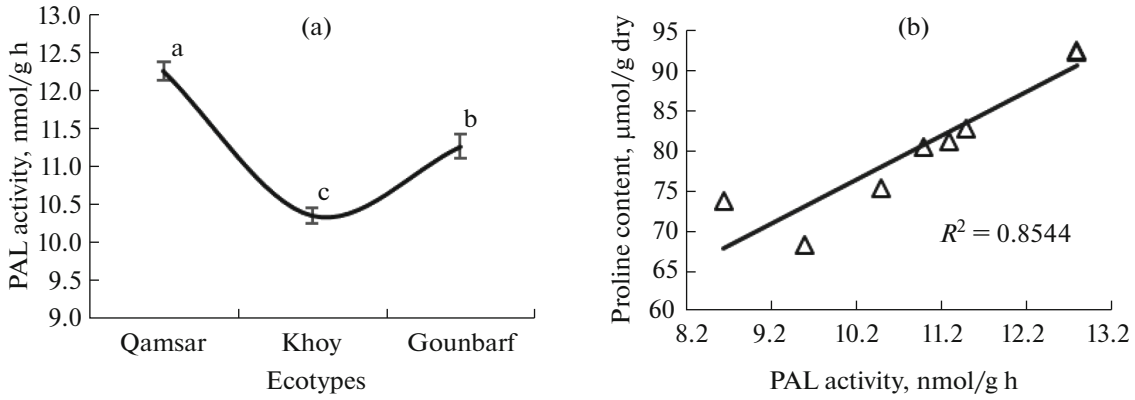

Fig. 4. PAL activity in different landraces of Damask rose (a) and correlation between PAL enzyme activity and proline content in Damask rose (b).

Phenolic Acids

HPLC analysis of Damask rose extract was done, and the chromatograms of compounds were evaluated (Fig. 5). Caffeic acid content was significantly affected by chelated fertilizers, chelated fertilizers concentrations, landrace, and interaction effect of landrace × chelated fertilizers concentration. However, the triple effect of chelated fertilizers × chelated fertilizers levels × landrace did not affect caffeic content significantly. Chelated fertilizers at 2 g L^{-1} in GN and control treatments in the QA landrace produced the highest and lowest content of caffeic acid, respectively (Table 2).

Moreover, the triple effect of treatments significantly affected gallic acid, chlorogenic acid, and coumaric acid. C-Zn at 2 g L^{-1} × GN landrace produced the highest content of gallic acid and the lowest content of gallic acid produced in 4 g L^{-1} C-Fe × KY landrace (Table 3). Furthermore, C-Fe at 2 g L^{-1} and control treatments in the QA landrace resulted in the highest and lowest content of chlorogenic acid, respectively. Coumaric acid production was the highest at control treatments × GN landrace, while control treatment in KY landrace resulted in the lowest coumaric acid (Table 3). Chelated fertilizers at 2 g L^{-1} in all landraces resulted in the highest caffeic acid content. C-Zn particle treatments resulted in more caffeic acid production compared to C-Fe (Table 4). The results indicated that chelated fertilizers at 2 g L^{-1} had a more effective impact on phenolic acids content including gallic, caffeic, coumaric, and chlorogenic acid.

Table 2. Interaction effect of landrace × chelated fertilizers concentrations on some secondary metabolites content in *Rosa damascena* extract

Landrace	Chelated fertilizers concentration, g L^{-1}	Secondary metabolites, μg L^{-1}			Proline, μmol g^{-1}dry wt
		Caffeic acid	Quercetin	Apigenin	
QA	0	2.59 ± 0.57	142.19 ± 9.4	2.77 ± 0.4	62.31 ± 4.8
	1	11.11 ± 2.46	179.4 ± 5.73	1.38 ± 0.3	57.62 ± 4.54
	2	11.4 ± 0.65	234.25 ± 6.7	3.41 ± 1.6	80.1 ± 3.6
	4	10.77 ± 1.61	182.7 ± 10.4	8.13 ± 0.98	96.53 ± 4.67
KY	0	2.09 ± 0.61	147.6 ± 4.9	2.73 ± 0.3	62.8 ± 1.53
	1	5.48 ± 0.39	135.5 ± 10.0	1.31 ± 0.16	59.9 ± 2.3
	2	12.63 ± 2.75	201.1 ± 12.8	4.76 ± 0.9	77.58 ± 2.03
	4	3.52 ± 0.99	131.0 ± 10.03	5.99 ± 1.3	93.01 ± 6.5
GN	0	7.76 ± 0.53	146.04 ± 2.49	2.9 ± 0.35	61.81 ± 2.7
	1	6.82 ± 1.83	189.3 ± 12.0	1.56 ± 0.3	56.5 ± 6.1
	2	15.37 ± 1.3	197.1 ± 9.5	9.9 ± 0.58	71.09 ± 6.5
	4	3.84 ± 0.69	93.46 ± 7.4	4.38 ± 1.02	91.3 ± 2.4

Landraces: QA, Qamsar; KY, Khoy; GN, Gounbarf. Values are means ± standard errors of one experiment with three biological replicates.

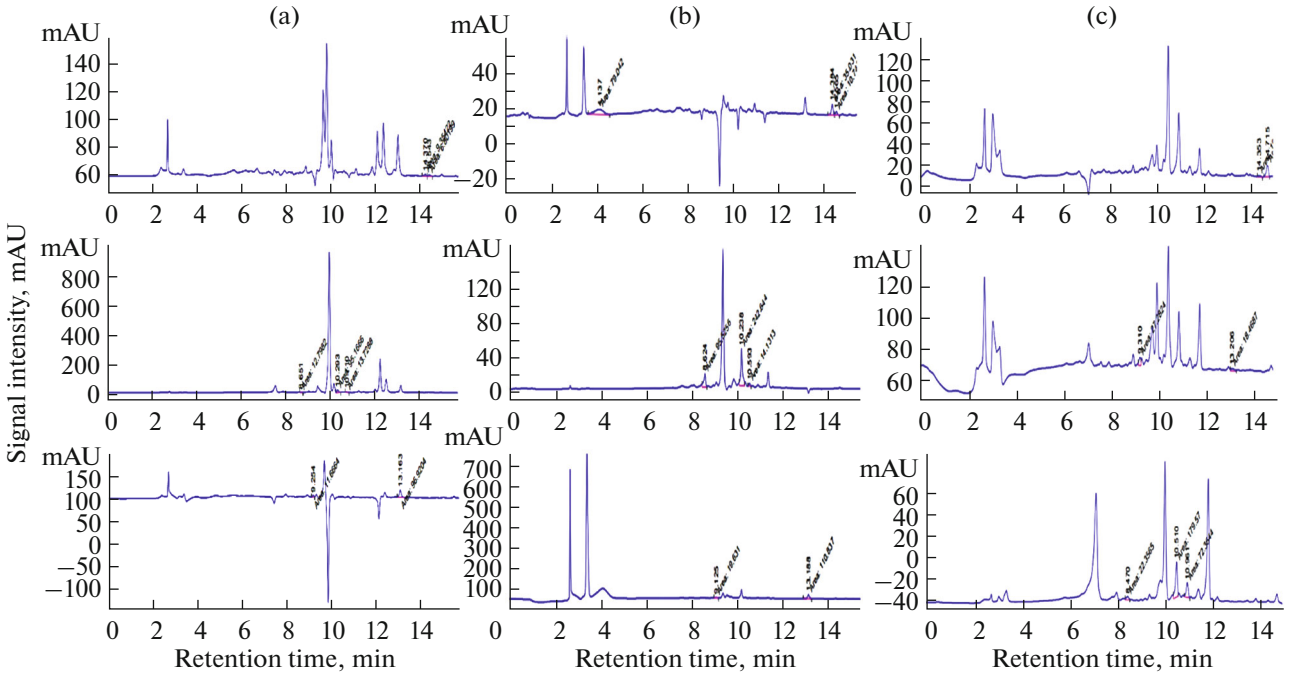

Fig. 5. HPLC chromatograms of secondary metabolites in different Damask rose landraces under control treatment. The chromatogram represents the presence of phenolics and flavonoid compounds in three landraces. (a) Qamsar; (b) Khoy; (c) Gounbarf.

Flavonoids

The contents of rutin, quercetin, kaempferol, and apigenin were significantly affected by chelated fertilizers, chelated fertilizers concentrations, landraces, and interaction effects among treatments. Rutin content was increased via the application of chelated fertilizers. The lowest rutin content was produced in control treatments in QA, where C-Fe at 2 g L^{-1} × QA landrace and C-Zn at 2 g L^{-1} × KY landrace produced the highest rutin content (Table 3). Application of chelated fertilizers accelerated rutin production. Further, the highest quercetin content was observed in 2 g L^{-1} C-Zn × QA landrace, followed by 4 g L^{-1} C-Fe × QA. C-Zn treatment increased quercetin content more efficiently compare to C-Fe (Table 4). Moreover, the highest and lowest kaempferol content was resulted in C-Zn at 4 g L^{-1} × QA and control treatments × QA landraces, respectively (Table 3). GN landrace at 2 g L^{-1} chelated fertilizers produced the highest apigenin content, and chelated fertilizers at 1 g L^{-1} concentration resulted in the lowest apigenin content in all landraces (Table 2).

Essential Oils

GC-MS analysis revealed a wide range of components in Damask rose oil. The most abundant compounds in the QA landrace were nonadecane (22.43%), citronellol (12.76%), geraniol (11.23%), and heneicosane (10.18%), respectively (Fig. 6, Table 5). Foliar application of C-Zn increased the heneicosane content and made the heneicosane the second major compound in QA landrace after nonadecane (Table 5). The sequence of EO compounds in C-Zn treatments on QA landrace was as follows: nonadecane, heneicosane, citronellol, and geraniol.

The highest content of citronellol, as a part of the most valuable compounds in Damask rose oil, was produced in control plants (Table 5). C-Fe at rate 1 g L^{-1} led to the highest content of Geraniol (13.03%) in QA landrace compared to other treatments, including C-Zn and C-Fe. In all C-Fe treatments on the QA landrace, geraniol content was higher than citronellol, whereas, in C-Zn treatments, the citronellol content was higher than geraniol.

The highest content of 2-phenethyl alcohol, which is in charge of the characteristic rose odor [22], in the QA landrace was produced in C-Fe at 4 g L^{-1}; 2.95%, followed by C-Fe at 2 g L^{-1}; 2.45% (Table 5). β-Myrcene and linalool were solely produced in control plants of QA landrace, and α-pinene did not produce in chelated fertilizers at 4 g L^{-1}. However, other treatments produced α-pinene, although the control treatment produced the highest content (Table 5).

In Khoy landrace, the main components in EO were nonadecane (26.43%), heneicosane (12.18%), Z-5-nonadecene (10.23%), and geraniol (7.32%), respectively (Table 6). Foliar application of C-Zn reduced the content of nonadecane and Z-5-nonadecene, while the geraniol content was increased (Table 6). In the C-Fe treatment, the highest content of geraniol and citronellol was produced in 2 g L^{-1}. This dosage led to the lowest nonadecane and heneicosane as well.

Table 3. Interaction effect of treatments on some secondary metabolites content in *Rosa damascena* extract

Landrace	Chelated fertilizers type	Concertation, g L^{-1}	Secondary metabolites, μg L^{-1}					
			Gallic acid	Chlorogenic acid	Coumaric acid	Rutin	Quercetin	Kaempferol
QA	C-Zn	0	1.36 ± 0.9	2.7 ± 0.92	4.22 ± 0.39	3.11 ± 0.58	12.55 ± 2.2	75.1 ± 3.1
		1	14.5 ± 2.1	3.24 ± 0.27	13.04 ± 0.34	7.11 ± 0.23	19.2 ± 0.9	182.3 ± 2.04
		2	14.79 ± 1.1	22.24 ± 1.43	13.59 ± 0.71	67.1 ± 1.02	24.3 ± 1.2	233.6 ± 6.44
		4	4.06 ± 0.2	28.52 ± 2.45	13.3 ± 0.74	66.1 ± 4.06	15.6 ± 1.1	245.8 ± 6.21
	C-Fe	0	1.31 ± 1.9	2.85 ± 0.63	8.0 ± 0.48	3.2 ± 0.68	13.5 ± 0.6	82.1 ± 2.48
		1	13.49 ± 1.1	7.41 ± 0.84	6.92 ± 2.69	13.8 ± 0.88	8.75 ± 1.3	176.4 ± 3.2
		2	16.57 ± 0.2	246.1 ± 16.2	14.81 ± 1.49	228.1 ± 10.1	19.9 ± 4.4	234.8 ± 4.8
		4	9.79 ± 0.5	11.65 ± 1.53	6.2 ± 1.47	9.75 ± 0.5	21.3 ± 2.9	119.5 ± 3.2
KY	C-Zn	0	9.24 ± 2.4	5.69 ± 0.67	8.66 ± 1.07	5.51 ± 0.79	6.7 ± 0.3	150.7 ± 3.6
		1	5.8 ± 0.9	6.43 ± 0.91	10.48 ± 0.82	8.58 ± 1.04	15.1 ± 4.4	167.3 ± 2.8
		2	22.4 ± 2.6	194.3 ± 6.36	11.35 ± 0.96	203.8 ± 14.1	17.2 ± 4.5	196.5 ± 3.76
		4	8.09 ± 1.1	7.05 ± 0.71	4.59 ± 1.47	15.8 ± 3.99	18.0 ± 3.7	182.9 ± 3.2
	C-Fe	0	9.36 ± 0.1	6.17 ± 0.05	3.59 ± 0.27	6.58 ± 0.11	10.0 ± 0.07	151.5 ± 1.05
		1	6.18 ± 0.9	12.99 ± 2.89	10.18 ± 0.62	39.5 ± 1.01	4.5 ± 0.5	103.8 ± 1.7
		2	1.69 ± 0.4	185.05 ± 5.0	11.08 ± 0.31	40.76 ± 1.32	4.1 ± 0.1	205.8 ± 2.78
		4	1.22 ± 0.4	18.1 ± 1.18	8.28 ± 0.21	28.85 ± 1.5	4.9 ± 0.4	99.1 ± 1.05
GN	C-Zn	0	4.87 ± 1.7	7.58 ± 0.14	24.23 ± 0.99	10.38 ± 0.37	4.8 ± 0.1	99.9 ± 2.44
		1	22.7 ± 2.5	29.78 ± 2.82	7.32 ± 1.27	11.2 ± 1.46	13.5 ± 0.3	201.8 ± 3.12
		2	26.6 ± 2.0	212.1 ± 13.6	9.18 ± 0.63	20.86 ± 2.05	9.4 ± 0.6	220.6 ± 2.49
		4	7.21 ± 0.9	10.58 ± 0.9	3.79 ± 0.35	11.79 ± 1.49	8.9 ± 1.1	109.2 ± 2.94
	C-Fe	0	4.97 ± 0.1	6.42 ± 0.08	25.42 ± 0.78	6.47 ± 0.04	6.4 ± 0.05	100.1 ± 1.72
		1	21.6 ± 0.9	14.47 ± 0.97	8.63 ± 0.5	32.09 ± 1.4	3.52 ± 0.5	176.8 ± 2.45
		2	3.17 ± 0.3	201.8 ± 19.3	16.84 ± 3.27	45.29 ± 1.08	13.3 ± 1.0	173.5 ± 1.3
		4	5.09 ± 0.1	6.23 ± 0.11	4.74 ± 0.52	27.67 ± 2.08	14.8 ± 0.5	77.6 ± 0.98

Landraces: QA, Qamsar; KY, Khoy; GN, Gounbarf. Values are means ± standard errors of one experiment with three biological replicates.

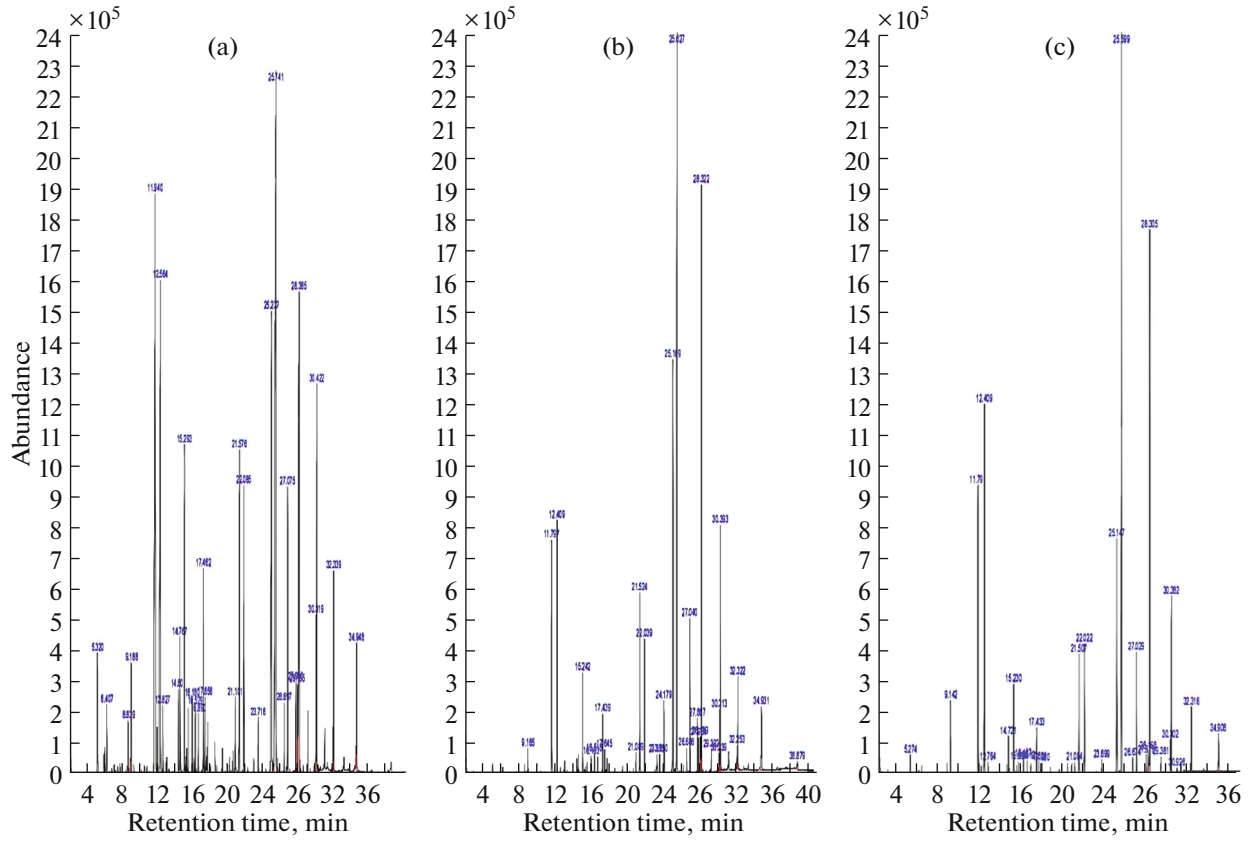

Fig. 6. GC-MS chromatograms of essential oil of different Damask rose landraces under control treatment. The chromatogram represents the abundance of different compounds in the three landraces of Damask rose. (a) Qamsar; (b) Khoy and (c) Gounbarf.

β-Myrcene and Linalool compounds were not observed in the KY landrace. However, these compounds were in the EO of the QA landrace (Table 6).

The most abundant components of GN landrace, were nonadecane (26.88%), heneicosane (13.5%), geraniol (12.02%), and citronellol (10.34%), respectively (Table 7). Application of C-Zn treatments reduced nonadecane, citronellol, and geraniol contents in GN landrace, except in 1 and 4 g L^{-1} C-Zn, which produced a higher content of citronellol and heneicosane compared to the control, respectively. In C-Fe treated GN landrace, the content of EO was changed and nonadecane, heneicosane, Z-5-nonadecene, and tricosane were the major compounds (Table 7). The highest level of total EO compounds in the GN landrace was produced in the control treatment, and chelated fertilizers application reduced the content of total EO.

Table 4. Effect of chelated fertilizers on caffeic acid and quercetin content in *Rosa damascena* flower extract

Chelated fertilizers type	Caffeic acid	Quercetin
C-Zn	9.5192[a]	183.19[a]
C-Fe	7.7867[b]	148.46[b]

Different letters in each column indicate significant differences determined using a Duncan's multiple range test ($P < 0.05$).

DISCUSSION

Accumulation of proline in plants is a general response to stress conditions. However, some studies have shown that proline accumulation in plants is not related to stress conditions and is a side product of plant responses. Furthermore, proline can act as a free radical scavenger and activator of detoxification pathways, stabilizer of subcellular structures, cytosolic pH buffer, and signalling molecule [23].

The decrease in proline content by application of chelated fertilizers at 1 g L^{-1} could be due to the positive effect of these micronutrients at lower concentrations on the reaction of Damask rose landraces to unfavourable environmental conditions. On the other hand, the increase of proline under C-Zn treatments can be due to the structural and functional role of Zn in the structure of ornithine aminotransferase enzyme, which is involved in proline synthesis. Furthermore, it seems that more increase in chelated fertilizers concentration has adversely affected growth

Table 5. Essential oil composition of *Rosa damascena* Mill. Qamsar landrace as affected by chelated fertilizers

Component	RI	R.T	%						
			Control	C-Zn, g L^{-1}			C-Fe, g L^{-1}		
			0	1	2	4	1	2	4
α-pinene	936	5.32	1.45	0.45	0.50	–	0.71	0.70	–
β-myrcene	992	6.41	0.54	–	–	–	–	–	–
Linalool	1097	8.78	0.6	–	–	–	–	–	–
2-Phenethyl alcohol	1115	9.16	1.43	0.67	1.01	2.14	1.36	2.45	2.95
Citronellol	1236		12.76	11.23	11.56	11.59	11.41	10.87	9.00
Neral	1242	12.11		0.44	0.31	–	–	0.39	–
Geraniol	1255	12.41	11.23	10.33	10.50	10.95	13.03	11.59	10.47
Geranial	1272	12.78	0.7	0.65	0.46	–	0.40	0.56	–
Citronellyl acetate	1351	14.56	0.33	–	–	–	–	–	–
Eugenol	1360	14.76	1.38	0.74	0.48	–	1.17	0.75	0.47
Geranyl acetate	1381	15.24	1.22	1.45	2.18	0.32	2.49	2.62	3.64
Trans-caryophyllene	1425	16.16	0.66	0.93	1.29	0.58	0.47	0.88	0.80
α-guaiene	1442	16.51	0.45	0.5	0.57	–	0.39	0.66	0.59
α-humulene	1460	16.89	0.49	0.53	0.60	–	–	0.45	0.46
Germacrene D	1486	17.43	1.77	1.79	1.79	0.97	1.34	1.68	1.41
N-pentadecane	1496	17.64	0.42	–	–	–	–	–	–
Cadinene	1639	20.43	–	–	–	–	0.36	–	–
α-eudesmol	1659	20.82	–	–	0.32	1.04	0.33	0.98	0.86
8-heptadecene	1673	21.08	0.8	0.63	0.37	0.31	0.32	–	–
Heptadecane	1696	21.52	2.76	2.22	1.96	2.40	3.13	1.70	1.87
1-heptadecanol	1720	22.03	3.33	3.38	3.98	2.17	3.30	4.70	5.56
Octadecane	1798	23.71	0.75	0.63	–	–	0.38	–	–
Z-5-nonadecene	1877	25.16	8.12	8.73	6.12	9.18	7.45	5.90	5.5
Nonadecane	1903	25.62	22.43	22.07	21.77	24.52	21.61	20.29	21.10
(E)-9-eicosene	1976	26.68	0.46	0.32	–	–	3.55	–	–
Eicosane	2000	27.04	2.87	2.5	2.77	2.56	2.56	2.57	2.90
Heneicosane	2097	28.32	10.18	12.23	16.62	16.57	11.65	13.70	15.08
Docosane	2199	29.39	–	–	0.53	0.47	0.40	0.44	0.54
9-tricosene	2291	30.31	1.14	1.76	0.92	1.51	0.75	0.93	1.03
Tricosane	2299	30.39	3.30	6.58	6.08	5.76	3.67	4.96	5.44
Pentacosane	2498	32.33	0.61	2.22	3.05	2.59	1.91	3.08	3.21
Heptacosane	2702	34.93	1.52	1.87	2.27	1.51	2.76	2.80	2.85
Total (%)			93.7	94.85	98.01	97.14	96.9	95.65	95.73

RI, Retention indices; RT, Retention time; "–" symbol, lack of synthesis of the compound in the EO.

conditions. In response to the unfavourable growth conditions, plants have increased the proline accumulation as an osmoregulator to maintain the water balance and mitigate lipid peroxidation [24]. The same results have been reported in *Triticale* [25], *Brassica rapa* [26], and *Dianthus carthusianorum* [27].

PAL enzyme catalyzes the first reaction in the shikimate/phenylpropanoid pathway. Changes in PAL activity level can directly impact downstream SMs production [28]. The results indicated that as proline production enhanced, the activity of the PAL enzyme increased (Fig. 4b). Here, chelated fertilizers treatment significantly affected PAL activity and the highest level of C-Fe and C-Zn led to the highest levels of PAL activity (Fig. 3b). It seems that higher concentrations of chelated fertilizers have limited the suitable growth conditions and proline content as an indicator

Table 6. Essential oil composition of *Rosa damascena* Mill. Khoy landrace as affected by chelated fertilizers

Component	RI	R.T	%						
			Control	C-Zn, g L^{-1}			C-Fe, g L^{-1}		
			0	1	2	4	1	2	4
α-pinene	936	5.32	–	0.45	0.65	–	–	0.65	0.65
2-Phenethyl alcohol	1115	9.16	0.6	0.67	1.2	1.92	1.11	0.97	0.72
Citronellol	1236	11.79	7.12	6.55	7.50	7.45	9.80	13.25	7.26
Neral	1242	12.11	–	–	–	–	–	–	–
Geraniol	1255	12.41	7.32	7.55	7.99	8.89	10.75	14.37	8.74
Geranial	1272	12.78	–	–	–	–	–	0.40	–
Citronellyl acetate	1351	14.56	–	–	–	–	0.40	0.41	–
Eugenol	1360	14.76	–	–	0.43	0.57	–	0.51	0.40
Geranyl acetate	1381	15.24	1.18	2.25	2.25	2.29	–	2.72	2.01
Trans-caryophyllene	1425	16.16	0.48	0.96	1.11	–	0.60	0.72	0.44
α-guaiene	1442	16.51	0.52	0.55	0.58	–	0.48	0.60	0.40
α-humulene	1460	16.89	–	–	0.44	–	0.36	0.40	0.29
Germacrene D	1486	17.43	1.22	1.32	1.47	1.36	1.51	1.34	1.64
N-pentadecane	1496	17.64	0.3	–	–	–	–	–	0.30
α-eudesmol	1659	20.82	–	–	0.59	1.07	1.12	0.34	–
8-heptadecene	1673	21.08	0.86	–	–	–	–	–	0.44
Heptadecane	1696	21.52	2.22	2.33	1.98	1.15	2.30	2.13	2.66
1-heptadecanol	1720	22.03	2.35	4.64	4.64	4.67	0.94	4.77	3.40
1-heptadecanol	1784	23.39	0.72	0.56	–	–	–	–	–
Octadecane	1798	23.71	0.47	0.54	–	–	–	–	–
Z-5-nonadecene	1877	25.16	9.23	8.88	7.58	7.77	6.52	6.91	6.74
Nonadecane	1903	25.62	26.43	23.56	22.42	21.31	22.12	20.91	22.10
(E)cyclododecene	1922	25.89	0.62	–	–	–	–	–	–
(E)-9-eicosene	1976	26.68	0.44	–	–	1.20	0.42	0.32	–
Eicosane	2000	27.04	4.56	2.72	3.07	3.58	3.33	2.58	2.44
Heneicosane	2097	28.32	12.18	15.76	16.74	16.95	18.13	13.16	17.61
Docosane	2199	29.39	0.7	0.86	0.62	0.46	0.48	0.38	0.60
9-tricosene	2291	30.31	1.20	1.85	1.12	0.99	1.46	0.91	1.42
Tricosane	2299	30.39	7.11	5.42	6.31	7.82	5.70	4.11	7.22
Pentacosane	2498	32.33	2.18	3.22	3.98	2.59	3.19	2.52	3.55
Heptacosane	2702	34.93	2.66	3.12	3.60	3.52	2.97	2.47	3.82
Total (%)			92.67	93.76	96.27	95.56	93.69	97.85	94.85

RI, Retention indices; RT, Retention time; "–" symbol, lack of synthesis of the compound in the EO.

of stress condition has increased (Fig. 3a), and finally enhance the PAL activity [29].

The differences among landraces in phenolic acids content could be due to genetic makeup and/or different responses of landraces to environmental conditions [30]. Moreover, nutrients availability can affect phenolics production in plants. Nitrogen treatment at 0.1 mM concentration led to produce the highest level of phenolics in three cultivars of basil (*Ocimum basilicum* L. 'Dark opal', 'Genovese', and 'Sweet tai'), compared to the higher levels of nitrogen treatments [31]. Foliar application of Zinc sulfate on *Vitis vinifera* 'Merlot' plants increased total phenolics content, which could be due to the increase in the expression of genes in the phenolics biosynthesis pathway [32]. Enrichment of *Pleurotus ostreatusand* and *Pleurotus eryngii* with selenium (Se) and Zn activated the synthesis of phenolic compounds and ascorbic acid. Furthermore, foliar application of N-Zn and N-Boron (N-B) on *Punica granatum* 'Ardestani' before full

Table 7. Essential oil composition of *Rosa damascena* Mill. Gounbarf landrace as affected by chelated fertilizers

Component	RI	R.T	%						
			Control	C-Zn, g L^{-1}			C-Fe, g L^{-1}		
			0	1	2	4	1	2	4
α-pinene	936	5.32	0.36	0.62	0.50	–	–	–	–
2-Phenethyl alcohol	1115	9.16	1.84	1.23	1.01	0.60	1.16	0.8	–
Citronellol	1236	11.79	10.34	12.12	11.56	10.27	6.31	6.11	5.28
Neral	1242	12.11	–	0.42	0.31	–	–	–	–
Geraniol	1255	12.41	12.02	11.02	10.50	9.09	5.91	5.18	3.01
Geranial	1272	12.78	0.25	0.51	0.46	–	–	–	0.27
Eugenol	1360	14.76	0.90	–	0.48	–	–	–	–
Geranyl acetate	1381	15.24	2.17	2.25	2.18	2.16	–	0.54	0.69
Trans-caryophyllene	1425	16.16	0.35	1.32	1.29	1.04	0.49	1.26	2.62
α-guaiene	1442	16.51	0.29	0.46	0.57	0.92	–	–	–
α-humulene	1460	16.89	–	0.52	0.60	0.67	–	–	–
Germacrene D	1486	17.43	1.19	1.32	1.79	2.35	1.19	1.04	1.08
N-pentadecane	1496	17.64	0.28	–	–	–	–	–	–
Cadinene	1639	20.43	–	–	–	–	–	–	0.46
α-eudesmol	1659	20.82	–	0.28	0.32	0.47	0.83	0.66	0.42
8-heptadecene	1673	21.08	0.32	0.51	0.37	–	–	0.36	0.53
Heptadecane	1696	21.52	3.40	2.11	1.96	1.52	2.14	2.02	1.53
1-heptadecanol	1720	22.03	3.87	2.47	3.98	4.18	1.37	0.87	–
Octadecane	1798	23.71	0.38	–	–	–	–	–	–
Z-5-nonadecene	1877	25.16	8.84	6.25	6.12	4.64	9.68	9.54	9.20
Nonadecane	1903	25.62	26.88	22.88	21.77	17.33	22.89	21.65	22.47
(E)-cyclododecene	1922	25.89	–	–	–	–	–	–	0.28
(E)-9-eicosene	1976	26.68	0.34	–	–	–	0.40	0.3	–
Eicosane	2000	27.04	2.83	2.75	2.77	2.75	2.88	2.5	2.02
Heneicosane	2097	28.32	13.50	16.43	16.62	16.98	18.32	18.55	19.54
Docosane	2199	29.39	0.32	0.38	0.53	0.75	0.56	0.61	0.71
9-tricosene	2291	30.31	0.76	1.24	0.92	0.85	1.91	1.95	2.01
Tricosane	2299	30.39	3.72	5.48	6.08	7.55	7.19	8.58	10.86
Pentacosane	2498	32.33	1.66	2.65	3.05	5.03	4.75	5.12	6.46
Heptacosane	2702	34.93	1.49	1.08	2.27	5.78	4.89	4.66	4.57
Total (%)			98.3	96.3	98.01	94.93	92.87	92.3	94.01

RI, Retention indices; RT, Retention time; "–" symbol, lack of synthesis of the compound in the EO.

bloom could slightly increase phenolics production, especially at higher levels of N-Zn and N-B. Meanwhile, diversity in the phenolic composition of different plant landraces has been reported in *lathyrus sativus, lens culinaris,* and *Cicer arietinum* [33].

The results of this study may be explained by the GDBH (Growth Differentiation Balance Hypothesis) framework, which explains when environmental conditions are good and adequate and balanced amounts of nutrients are available, plant growth conditions will improve, however when the imbalance of nutrients or unfavourable environmental conditions exists, the growth allocation for the plant is limited, and production of SMs enhances [31].

Rutin as a flavonoid compound with potent antioxidant activity ubiquitously synthesizes in plants to protect against oxidative stresses. Rutin is a quercetin-based flavonoid known for its health-promoting attributes and efforts have been made to increase its quantity in plant tissues and improve water solubility to be more used for therapeutic processes [30]. Quercetin and its derivatives are polyphenol compounds synthe-

sized within plant tissues and have various pharmacological effects [3]. Likewise, kaempferol and apigenin are found in natural products and protect plant tissues against oxidative stresses [30]. This study showed the different patterns of phenolics production among Damask rose landraces (Tables 1, 2).

Minerals availability can affect flavonoid production in plants. A reverse relationship was found between nitrogen and phosphorus content with flavonoids production in *Arabidopsis thaliana*, tomato seedlings, and *Hypericum perforatum* L. [34], which is consistent with the findings of this study (Tables 2, 3). Results of this research revealed that high proline content (Table 2) as well as high PAL enzyme activity (Figs. 3b, 4a) in higher levels of chelated fertilizers concentration have led to the production of higher levels of flavonoids compounds, however, PAL enzyme in shikimate/phenylpropanoid pathway is not the sole enzyme affecting flavonoids content and other enzymes such as PAL, CHS, CHI, and FLS can directly and/or indirectly affect flavonoid synthesis [35].

In QA and KY landraces, chelated fertilizers treatments increased the total contents of EO. However, in the GN landrace the content of EO was reduced via the application of chelated fertilizers. Mirzaei et al. [36] reported that the best ratio of Citronellol/Geraniol should be in the range of 1.25–1.30 to full fit the demand of the perfumery industry. None of studied landraces fill this demand and application of C-Zn and C-Fe could not maintain the above-mentioned ratio, although chelated fertilizers at GN landrace could slightly increase the suggested ratio. These results indicate that the application of nutrient elements can change EO composition in Damask rose and more investigations on this target can help us to maintain the preferred EOs for the perfumery industry.

Zinc treatment at different concentrations affected the EO content in *Pelargonium graveolens*. Further, chelated Fe-EDTA and Zn-EDTA significantly affected the EO composition in *Ocimum basilicum*. Moreover, Fe and Zn treatments as ferrous and zinc sulfate increased the EO content in *Matricaria chamomilla* [13]. Chelated fertilizers treatment increased the total content of rose EO in QA and KY landraces, which is consistent with reports on EO enhancement via the application of micronutrients. This could be due to the role of micronutrients, especially Fe and Zn, as metal components of some enzymes, in activating their functions [37]. In addition, chelated fertilizers can alleviate the reactive oxygen species and oxidative stress and improve photosynthetic processes, nucleic acid assimilation, cell division, accumulation of total carbohydrates, and finally, the total content of rose EO [17]. Previous studies have shown that any increase in total plant carbohydrates, the most important direct organic product of photosynthesis, will be employed in the biosynthesis of EO, consequently increasing the production of EO [13]. However, the total content of EO was reduced in the GN landrace via the application of C-Zn and C-Fe, which may be due to the genetic diversity and different responses of plants to the application of nutrients [38]. Vieira et al. [39] reported that genetic variation might affect the segregation of some compounds in EO composition. The diversity among EO composition in different plant cultivars and accessions have been previously reported.

CONCLUSIONS

The present study indicated that the foliar application of chelated fertilizers (C-Zn and C-Fe) could effectively change the secondary metabolite content and essential oil compositions in different Damask rose landraces. Increasing the chelated fertilizers concentration to 4 g L^{-1} increased proline content and PAL enzyme activity. In addition, a positive correlation was observed between proline content and PAL activity. The HPLC results revealed that the most abundant compounds in the QA landrace were nonadecane, citronellol, geraniol, and heneicosane, respectively. In the QA landrace, C-Zn treatments at the rates 2 and 4 g. L^{-1} produced the highest content of quercetin and kaempferol, respectively. Also, C-Fe (2 g L^{-1}) led to the highest rutin content. Variations in secondary metabolites content and essential oil compositions in different Damask rose landraces may be due to the change in the activity of some enzymes, such as PAL, downstream enzymes, and other enzymes that are involved in the shikimate/phenylpropanoid pathway and acetate/mevalonate pathway, respectively. These findings suggest that foliar chelated fertilizers application on the QA landrace can enhance the amount of anti-cancer and antioxidant compounds. Therefore, these treatments are suitable for increasing the SMs content of the QA landrace in therapeutic processes.

ACKNOWLEDGMENTS

We thank University of Mohaghegh Ardabili for the financial support of this study.

FUNDING

This research was funded by University of Mohaghegh Ardabili.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This work does not contain any studies involving human and animal subjects.

CONFLICT OF INTEREST

The authors of this work declare that they have no conflicts of interest.

REFERENCES

1. Teixeira, B., Marques, A., Ramos, C., Batista, I., Serrano, C., Matos, O., Neng, N.R., Nogueira, J.M.F., Saraiva, J.A., and Nunes, M.L., European pennyroyal (*Mentha pulegium*) from Portugal: Chemical composition of essential oil and antioxidant and antimicrobial properties of extracts and essential oil, *Ind. Crops Prod.*, 2012, vol. 36, no. 1, pp. 81–87. https://doi.org/10.1016/j.indcrop.2011.08.011
2. Sreenivasulu, N. and Fernie, A.R., Diversity: Current and prospective secondary metabolites for nutrition and medicine, *Curr. Opin. Biotechnol.*, 2022, vol. 74, pp. 164–170. https://doi.org/10.1016/j.copbio.2021.11.010
3. Kanani, M., Chamani, E., Shokouhian, A.A., and Torabi-Giglou, M., Plant secondary metabolism and flower color changes in Damask rose at different flowering development stages, *Acta Physiologiae Plant.*, 2021, vol. 43, no. 4, pp. 1–10. https://doi.org/10.1007/s11738-021-03200-w
4. Kanani, M., Chamani, E., Shokouhian, A.A., and Torabi-Giglou, M., Essential oil content and composition in various ecotypes of Damask rose from different ecological regions, *Acta Sci. Polonorum Hortorum Cultus*, 2021, vol. 20, no. 1, pp. 61–69. https://doi.org/10.24326/asphc.2021.1.6
5. Chamani, E., Irving, D.E., Joyce, D.C., and Arshad, M., Studies with thidiazuron on the vase life of cut rose flowers, *J. Appl. Horticulture*, 2006, vol. 08, no. 1, pp. 42–44. https://doi.org/10.37855/jah.2006.v08i01.10
6. Galal, T.M., Majrashi, A., Al-Yasi, H.M., Farahat, E.A., Eid, E.M., and Ali, E.F., Taif's rose (*Rosa damascena* Mill var. trigentipetala) wastes are a potential candidate for heavy metals remediation from agricultural soil, *Agriculture*, 2022, vol. 12, no. 9, p. 1319. https://doi.org/10.3390/agriculture12091319
7. Ali, E.F., Al-Yasi, H.M., Majrashi, A., Farahat, E.A., Eid, E.M., and Galal, T.M., Chemical and nutritional characterization of the different organs of Taif's rose (*Rosa damascena* Mill. var. trigintipetala) and possible recycling of the solid distillation wastes in Taif city, *Saudi Arabia. Agric.*, 2022, vol. 12, no. 11, p. 1925. https://doi.org/10.3390/agriculture12111925
8. Boskabady, M.H., Shafei, M.N., Saberi, Z., and Amini, S., Pharmacological effects of *Rosa damascena, Iran. J. Basic Med. Sci*, 2011, vol. 14, no. 4, pp. 295–307.
9. Baydar, N.G. and Baydar, H., Phenolic compounds, antiradical activity and antioxidant capacity of oil-bearing rose (*Rosa damascena* Mill.) extracts, *Ind. Crops Prod.*, 2013, vol. 41, pp. 375–380. https://doi.org/10.1016/j.indcrop.2012.04.045
10. Rasouli, O., Ahmadi, N., Rashidi Monfared, S., and Sefidkon, F., Physiological, phytochemicals and molecular analysis of color and scent of different landrace of *Rosa damascena* during flower development stages, *Sci. Hortic. (Amsterdam)*, 2018, vol. 231, pp. 144–150. https://doi.org/10.1016/j.scienta.2017.12.010
11. Mahmoud, A.W.M. and Taha, S.S., Main sulphur content in essential oil of *Eruca sativa* as affected by nano iron and nano zinc mixed with organic manure, *Agric*, 2018, vol. 64, no. 2, pp. 65–79. https://doi.org/10.2478/agri-2018-0007
12. Solanki, P., Bhargava, A., Chhipa, H., Jain, N., and Panwar, J., *Nano-fertilizers and their smart delivery system, Nanotechnologies in Food and Agriculture*, Rai, M., Ribeiro, C., Mattoso, L., and Duran, N., Eds., Cham: Springer, 2015, pp. 81–101.
13. Ali, E.F., Issa, A.A., Al-Yasi, H.M., Hessini, K., and Hassan, F.A.S., The efficacies of 1-methylcyclopropene and chitosan nanoparticles in preserving the postharvest quality of Damask rose and their underlying biochemical and physiological mechanisms, *Biology*, 2022, vol. 11, no. 2, p. 242. https://doi.org/10.3390/biology11020242
14. Vafa, Z.N., Sirousmehr, A.R., Ghanbari, A., Khammari, I., and Falahi, N., Effects of nano zinc and humic acid on quantitative and qualitative characteristics of savory (*Satureja hortensis* L.), *Int. J. Biosci.*, 2015, vol. 6, no. 3, pp. 124–136. https://doi.org/10.12692/ijb/6.3.124-136
15. Jabarpour, S., Zehtab, S., Alyari, H., Javanshir, A., and Shakiba, M.R., Effects of sowing time and plant density on yield and essential oil production of medicinal plant, peppermint (*Mentha piperita* L.), *Plant Sci. Res.*, 2013, vol. 1, no. 4, pp. 24–26. https://doi.org/10.22067/jag.v5i4.32997
16. Zakerin, H.R., Shirani Rad, A.H., Seifzadeh, S., Valadabadi, S.A., and Mostashari Mohasses, M., Evaluation the effect of Zn, Fe foliar application and variety on quality and physiological characteristics of canola (*Brassica napus* L.) under different ranges water deficit stress, *Adv. Environ. Biol.*, 2014, vol. 8, no. 1, pp. 255–260. http://www.aensiweb.com/aeb/2014/255-260.pdf.
17. El-Sonbaty, A.E., Farouk, S., Al-Yasi, H.M., Ali, E.F., Abdel-Kader, A.A.S., and El-Gamal, S.M.A., Enhancement of rose scented geranium plant growth, secondary metabolites, and essential oil components through foliar applications of iron (nano, sulfur and chelate) in alkaline soils, *Agronomy*, 2022, vol. 12, no. 9, p. 2164. https://doi.org/10.3390/agronomy12092164
18. Bates, L.S., Waldren, R.P., and Teare, I.D., Rapid determination of free proline for water-stress studies, *Plant Soil*, 1973, vol. 39, pp. 205–207. https://doi.org/10.1007/BF00018060
19. Wang, J.W., Zheng, L.P., Wu, J.Y., and Tan, R.X., Involvement of nitric oxide in oxidative burst, phenylalanine ammonia-lyase activation and Taxol production induced by low-energy ultrasound in *Taxus yunnanensis* cell suspension cultures, *Nitric Oxide*, 2006, vol. 15, no. 4, pp. 351–358. https://doi.org/10.1016/j.niox.2006.04.261
20. Marks, S.C., Mullen, W., and Crozier, A., Flavonoid and hydroxycinnamate profiles of English apple ciders, *J. Agric. Food Chem.*, 2007, vol. 55, no. 21, pp. 8723–8730. https://doi.org/10.1021/jf071155u
21. Clevenger, J.F., Apparatus for the determination of volatile oil, *J. Pharm. Sci.*, 1928, vol. 17, no. 4, pp. 345–349. https://doi.org/10.1002/jps.3080170407
22. Gunes, E., Turkey rose oil production and marketing: A review on problem and opportunities, *J. Appl. Sci.*, 2005, vol. 5, no. 10, pp. 1871–1875. https://doi.org/10.3923/jas.2005.1871.1875

23. Ashraf, M. and Foolad, M.R., Roles of glycine betaine and proline in improving plant abiotic stress resistance, *Environ. Exp. Bot.*, 2007, vol. 59, no. 2, pp. 206–216. https://doi.org/10.1016/j.envexpbot.2005.12.006
24. Tripathi, B.N. and Gaur, J.P., Relationship between copper- and zinc-induced oxidative stress and proline accumulation in *Scenedesmus* sp., *Planta*, 2004, vol. 219, pp. 397–404. https://doi.org/10.1007/s00425-004-1237-2
25. Torabian, S., Zahedi, M., and Khoshgoftarmanesh, A., Effect of foliar spray of zinc oxide on some antioxidant enzymes activity of sunflower under salt stress, *J. Agric. Sci. Technol.*, 2016, vol. 18, pp. 1013–1025.
26. Jan, S.A., Shinwari, Z.K., and Rabbani, M.A., Agro-morphological and physiological responses of *Brassica rapa* ecotypes to salt stress, *Pak. J. Bot.*, 2016, vol. 48, no. 4, pp. 1379–1384.
27. Wójcik, M. and Tukiendorf, A., Accumulation and tolerance of lead in two contrasting ecotypes of *Dianthus carthusianorum, Phytochemistry*, 2014, vol. 100, pp. 60–65. https://doi.org/10.1016/j.phytochem.2014.01.008
28. Kanani, M. and Nazarideljou, M.J., Methyl jasmonate and a-aminooxi-b-phenyl propionic acid as PAL enzyme promoter and inhibitor affected longevity and floral scent of cut tuberose (*Polianthes tuberosa* L.), *Hortic. Environ. Biotechnol.*, 2017, vol. 58, pp. 136–143.
29. Wada, K.C., Mizuuchi, K., Koshio, A., Kaneko, K., Mitsui, T., and Takeno, K., Stress enhances the gene expression and enzyme activity of phenylalanine ammonia-lyase and the endogenous content of salicylic acid to induce flowering in pharbitis, *J. Plant Physiol.*, 2014, vol. 171, no. 11, pp. 895–902. https://doi.org/10.1016/j.jplph.2014.03.008
30. Chamani, E., Wagstaff, C., and Kanani, M., Phenolics pattern of cut H_3O rose flowers during floral development, *Sci. Hortic. (Amsterdam)*, 2020, vol. 271, p. 109460. https://doi.org/10.1016/j.scienta.2020.109460
31. Nguyen, P.M. and Niemeyer, E.D., Effects of nitrogen fertilization on the phenolic composition and antioxidant properties of basil (*Ocimum basilicum* L.), *J. Agric. Food Chem.*, 2008, vol. 56, no. 18, pp. 8685–8691. https://doi.org/10.1021/jf801485u
32. Song, C.Z., Liu, M.Y., Meng, J.F., Chi, M., Xi, Z.M., and Zhang, Z.W., Promoting effect of foliage sprayed zinc sulfate on accumulation of sugar and phenolics in berries of *Vitis vinifera* cv. Merlot growing on zinc deficient soil, *Molecules*, 2015, vol. 20, no. 2, pp. 2536–2554. https://doi.org/10.3390/molecules20022536
33. Fratianni, F., Cardinale, F., Cozzolino, A., Granese, T., Albanese, D., Di Matteo, M., Zaccardelli, M., Coppola, R., and Nazzaro, F., Polyphenol composition and antioxidant activity of different grass pea (*Lathyrus sativus*), lentils (*Lens culinaris*), and chickpea (*Cicer arietinum*) ecotypes of the Campania region (Southern Italy), *J. Funct. Foods*, 2014, vol. 7, pp. 551–557. https://doi.org/10.1016/j.jff.2013.12.030
34. Azizi, M. and Dias, A., Nitrogen and phosphorus fertilizers affect flavonoids contents of St. John's Wort (*Hypericum Perforatum* L.), *Proc. Fourth Int. Iran & Russia Conf.*, 2004, pp. 458–462.
35. Gupta, N., Sharma, S.K., Rana, J.C., and Chauhan, R.S., Expression of flavonoid biosynthesis genes vis-à-vis rutin content variation in different growth stages of *Fagopyrum* species, *J. Plant Physiol.*, 2011, vol. 168, no. 17, pp. 2117–2123. https://doi.org/10.1016/j.jplph.2011.06.018
36. Mirzaei, M., Sefidkon, F., Ahmadi, N., Shojaeiyan, A., and Hosseini, H., Damask rose (*Rosa damascena* Mill.) essential oil is affected by short-and long-term handling, *Ind. Crops Prod.*, 2016, vol. 79, pp. 219–224. https://doi.org/10.1016/j.indcrop.2015.11.011
37. Rengel, Z., Cakmak, I., and White, P.J., *Marschner's Mineral Nutrition of Plants*, Academic, 2023, 4th ed.
38. Golparvar, A.R. and Bahari, B., Effects of phenological stages on herbage yield and quality/quantity of oil in garden thyme (*Thymus vulgaris* L.), *J. Med. Plant Res.*, 2011, vol. 5, no. 25, pp. 6085–6089. https://doi.org/10.5897/JMPR11.1136
39. Vieira, R.F., Grayer, R.J., Paton, A., and Simon, J.E., Genetic diversity of *Ocimum gratissimum* L. based on volatile oil constituents, flavonoids and RAPD markers, *Biochem. Syst. Ecol.*, 2001, vol. 29, no. 3, pp. 287–304. https://doi.org/10.1016/S0305-1978(00)00062-4

Publisher's Note. Pleiades Publishing remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.