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ORIGINAL PAPER

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FOLIAR APPLICATION OF POTASSIUM AND ZINC ENHANCES THE PRODUCTIVITY AND VOLATILE OIL CONTENT OF DAMASK ROSE (*Rosa damascena* Miller var. *trigintipetala* Dieck)

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ABSTRACT

Potassium (K) levels are decreasing worldwide in agricultural soils, and K deficiency is becoming a major issue. Study on damask rose response to K application is scarce. Furthermore, despite its importance in the cell division, photosynthesis and protein synthesis, there is a lack of published reports on plant responses to zinc (Zn) application. Further research is required to understand the damask rose's response to both elements. This study investigated the effects of K and Zn foliar application on the vegetative growth, flower yield, and volatile oil content and composition of damask rose. K and Zn nutrition was applied either individually or combined as K_2SO_4 and $ZnSO_4$ at 0.5 or 1.0%. Foliar application of K_2SO_4 and $ZnSO_4$ was applied with a manual pump four times in each growing season, the first at the beginning of stem elongation and leaf formation, and then at two-weekly intervals. Results showed that K and/or Zn treatments significantly improved the growth characters, flower yield, relative water content (RWC), stomatal conductance, and essential oil content and composition such as linalool, nerol, citronellol, geraniol, and nonadecane. The chlorophyll content, total soluble sugars (TSS), and protein content also increased, but free amino acid content decreased, suggesting that the distribution of nitrogenous compounds (between amino acids and proteins) and their transformation were influenced by K and Zn supply. Individual applications of K or Zn increased the N, P, K, and Zn contents in damask rose leaves, relative to the control, which increased further with combined applications of K and Zn. Results suggest that foliar application of K and/or Zn could be part of the damask rose fertilization program to provide plants with the optimum level of nutrition for improving the quantity and quality of flowers and essential oil yields.

Key words: chlorophyll, flower yield, growth, nutrients, protein, soluble sugars

INTRODUCTION

Damask rose (*Rosa damascena* Miller var. *trigintipetala* Dieck) is an aromatic plant that can be economically cultivated for its volatile oil. Damask rose is an outstanding species of the *Rosa* genus in the Rosaceae family that is principally cultivated for perfume, medicine, food industries, and as ornamental plants in parks, gardens, and homes [Jabbarzadeh and Khosh-Khui 2005]. Several products of this aromatic plant including rose oil, rose concrete, rose absolute, and rose water are widely used for perfume and in the pharmaceutical, cosmetic, and food industries [Ali et al. 2014, Abdel-Hameed et al. 2016]. Damask rose is used for

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its antioxidant, anti-diabetic, anti-HIV, anti-bacterial, and anti-inflammatory activities as well as its cardiotonic effect [Baydar and Baydar 2013, Boskabady et al. 2014, Baniasad et al. 2015]. The volatile oil from damask rose is among the most expensive oils in markets worldwide due to the low oil content in petals [Baydar and Baydar 2005]. The major constituents of damask rose oil are acyclic monoterpene alcohols (mainly citronellol, E-geraniol, and nerol) and hydrocarbons (mainly nonadecane, nonadecene, and heneicosane) [Mohamadi et al. 2011, Hassan and Ali 2018, Al-Yasi et al. 2020]. Genetic and environmental factors can improve flower productivity and oil yield in rose [Danyaie et al. 2011, Pal, 2013]. About 175 000 species of plants on earth are grown in tropical and subtropical areas. During evolution, they could not develop the ability to withstand low temperatures [Pal et al. 2014].

Additionally, nutrient availability plays an important role in this regard [Ahmad et al. 2010, Younis et al. 2013, Kumar et al. 2016a, Secco et al. 2017]. Due to insufficient information and technical skill on the optimum levels of various nutrients, producers are unable to maximize the production and quality of damask rose. Macro and micronutrient treatments play a crucial role in this respect [Khoshgoftarmanesh et al. 2008, Huang et al. 2019]. Nutrient management is important for optimizing economic and environmental sustainability. Potassium deficiency, as a macronutrient, in plants is a major issue in many countries, and the K status of agricultural soils is declining worldwide [Tan et al. 2012]. There is a lack of published reports on plant responses to K application. Despite being added as fertilizer, K balance can decline significantly over time in some soils [Krauss 2003]. Potassium supplementation is required in certain soils depending on soil type, nutrient competition, and water availability [Zörb et al. 2014].

Potassium is an important inorganic cation that plays several roles in plant physiological processes including enzyme activity, osmotic potential maintenance, stomatal conductance, photosynthesis maintenance, water homeostasis, growth turgor, and solute transport [Cakmak 2005, Benito et al. 2014, Oosterhuis et al. 2014]. The role of K in improving growth characters and the yield quantity and quality in other crops has been reported in hemp [Finnan and Burke 2013] and cotton [Tsialtas et al. 2016]. *Jatropha curcas* L. plants fertilized with K produced higher fruit and seed yields than the control, along with a two-fold increase in oil production [Omar et al. 2014]. Application of K can also reduce free amino acid content and increase protein and carbohydrate contents in maize [Qu et al. 2011] and cotton [Wang et al. 2012, Hu et al. 2015, 2016]. Additionally, foliar K application affected leaf gas exchange in cotton by increasing CO_2 assimilation rate and stomatal conductance, and thereby reducing leaf temperature and increasing leaf water potential [Tsialtas et al. 2016].

Micronutrients including Zn also play a protagonist role in improving growth and yield quality in several crops [Sawan et al. 2001]. Small deficiencies in micronutrients can disturb physiological and metabolic processes in plants [Brady and Ray 2002]. Furthermore, micronutrients are a vital component of plant enzyme systems [Massoud et al. 2005]. The total herb and volatile oil yields of palmarosa (*Cymbopogon martinii*) increased with micronutrient application [Rao and Rajput 2011]. Zinc is essential for improving plant growth and production [Parker et al. 1992]. Foliar application of Zn increased flower numbers, flower weight and flower yield in *Rosa damascena* Mill plant, and markedly changed the qualitative and quantitative composition of the volatile oil [Kumar et al. 2016a]. In addition, foliar application of Zn significantly increased plant height in maize [Saeed and Amin 2019] and dry matter accumulation in rice [Khan et al. 2012] relative to their respective control. Several studies in medicinal and aromatic plants have shown that Zn improves vegetative growth and yields of *Nigella sativa* L. [Mousa et al. 2001] and *Coriandrum sativum* L. [Said-Al Ahl and Omer 2009]. Zn is a microelement that acts as a metal component of different enzymes or regulatory cofactors, and for auxin synthesis, cell division, photosynthesis, protein synthesis, and maintenance of membrane structure and function [Marschner 1995].

Available field research on the response of damask rose to K and Zn application is limited. More research is needed to understand its response to K and Zn application. Consequently, this study investigated the effects of K and Zn foliar nutrition on growth, flower yield, and the volatile oil content of damask rose.

MATERIALS AND METHODS

Plant materials and treatments. A two-year (2017 and 2018) field experiment was undertaken on a private farm in the Taif region, Saudi Arabia to investigate the impact of potassium (K) and zinc (Zn) foliar application on damask rose (*Rosa damascena* Miller var. *trigintipetala* Dieck). The soil consisted of 80.05% sand, 7.21% silt, and 12.74% clay, and had a pH of 8.01, EC of 2.06 dsm^{-1}, 0.15% organic matter, 0.81% total $CaCO_3$, 3.31 meq L^{-1} Na^+, , 42.89 meq L^{-1} Ca^{2+}, 49.08 meq L^{-1} SO_4^{2-}, 2.13 meq L^{-1} HCO_3, 0.59 meq L^{-1} Cl^-, 0.21% N^+, 0.041% PO_4^{3-}, and 0.054% K^+.

Five-year-old damask rose bushes (2500 hill/ha) were used for the seven treatments (T1: control, plants sprayed with water; T2: 0.5% K_2SO_4; T3: 1% K_2SO_4; T4: 0.5% $ZnSO_4$; T5: 1% $ZnSO_4$; T6: 0.5% K_2SO_4 + 0.5% $ZnSO_4$; T7: 1% K_2SO_4 + 1% $ZnSO_4$). The experimental design was a randomized Randomized compete block design (RCBD) with four replicates. Foliar application of K_2SO_4 and $ZnSO_4$ was applied with a manual pump four times in each growing season, the first at the beginning of stem elongation and leaf formation, and then at two-weekly intervals. A constant dose of N (75 N ha^{-1} as ammonium nitrate) and P (100 P ha^{-1} as single super-phosphate) fertilizer was added to all bushes. A drip irrigation system was used, and the block were manually weeded.

Growth and flower yield parameters. Plant height (cm), main and secondary branch numbers per hill, and dry weights (%) of leaf and stem were registered by the end of the flowering season. In order to measure the total flowers yield per hill, flowers from each hill were periodically harvested, counted, and fresh weight (FW) registered.

Relative water content. Leaf relative water content (RWC) was measured on harvested leaves using the method of Weatherley [1950] with the following equation: $(W_{fresh} - W_{dry}) / (W_{turgid} - W_{dry}) \times 100$, where W_{fresh} is sample FW, W_{turgid} is sample turgid weight after being saturated with distilled water for 24 h at 4°C, and W_{dry} is the oven-dried sample weight (70°C for 48 h).

Stomatal conductance. Stomatal conductance (mol H_2O m^{-2} s^{-1}) was measured on intact leaves prior to harvest using a Delta T AP4 leaf porometer (UK).

Essential oil content and composition analysis. Volatile oil was extracted from flowers using the hydro-distillation method in a Clevenger-type apparatus according to British Pharmacopoeia (1963). Flowers were harvested in the early morning and immediately brought to the laboratory for distillation. For each treatment, 1000 g flowers were hydro-distilled with 3 L of water in the 5 L capacity Clevenger-type apparatus for 6 h according to Kumar et al. [2016a] with some modification. At the end of distillation, the volatile oil percentage was estimated as (v/w, %) and oil yield per hill and per ha was calculated. The obtained volatile oil was dehydrated over anhydrous sodium sulfate and stored at 4°C in the refrigerator until GC-MS analysis. The oil sample solutions and standards preparation are described in Abdel-Hameed et al. [2016]. Volatile oil samples were collected using a Varian GC CP-3800 and MS Saturn 2200 equipped with a Factor Four capillary column (VF-5 ms 30 × 0.25 mm ID and film thickness 0.25 μm). An electron ionization system with ionization energy of 70 eV was used for GC-MS detection. The peaks of volatile oil components were identified by comparing their retention indices and mass spectrum with those of the standards, the NIST library of the GC-MS system, and published data.

Nutrient elements determination. Total N content was measured using the micro-Kjeldahl digestion method described by Nelson and Sommers [1973]. Phosphorus was colorimetrically determined using a spectrophotometer (Pharmacia, LKB-Novaspec II) and K was measured using a flame photometer. The Zn content in leaves was determined using the method of Fuwa et al. [1964]. Briefly, a 1 g leaf sample was digested in a 250 mL glass tube with 15 mL of nitric acid (HNO_3) at 140°C for 2 h. The contents were cooled to room temperature and directly dried. The sample was then treated with 3 mL of $HClO_4$ for further oxidation for 30 min at 240°C. The sample was diluted with 10 mL of distilled water, filtered, and made up to 100 mL with distilled water. Zinc analysis of was performed using atomic absorption.

Chlorophyll content. Random samples of fresh leaves were taken for chlorophyll determination. Total chlorophyll content (mg g^{-1} FW) was measured as described by Sadasivam and Manickam [1992] using a spectrophotometer (Pharmacia, LKB-Novaspec II).

Total soluble sugars. Total soluble sugars (TSS, %) were determined in leaf samples according to the method of Dubois et al. [1956].

Free amino acid and soluble protein contents. Free amino acids were first extracted using the method of Ruiz and Romero [2002]. Briefly, a 0.5 g fresh leaf sample was crushed in 5 mL of cold phosphate buffer (50 mM KH_2PO_4, pH 7), and centrifuged at 12,000 × g for 15 min. The obtained supernatant was used for free amino acid and soluble protein analyses. The ninhydrin method was used to determine the total free amino acid content according to Yemm et al. [1955] and expressed as mg g^{-1} FW. The Brilliant Blue G-250 regent was used to determine soluble protein content [Bradford 1976] using bovine serum albumin (BSA) as a standard.

Statistical analysis. This experiment was repeated twice; the obtained results were homogenous and, therefore, pooled ($n = 8$) for analysis. Analysis of variance (ANOVA) was performed, and data analyzed using SPSS 13.3 program with the means compared by Duncan's multiple range test at the $P \leq 0.05$ level. The results were expressed as mean values (±SD).

RESULTS AND DISCUSSION

Growth characteristics. Plant height, main and secondary branch numbers, and leaf and stem dry weights of damask rose increased with K and/or Zn application, relative to the control plants, and the greatest improvements occurred in the combined treatments (T6 and T7) (Tab. 1). Such increases in growth characteristics are likely due to the applied K promoting the activity of some enzymes and increasing the translocation of assimilates and protein synthesis [Khetsha and Sedibe 2015, Saeed and Amin 2019]. In addition, Zn enhances photosynthetic and other metabolic activities that increase various plant metabolites required for cell division and elongation [Younis et al. 2013].

Moreover, the positive effects of nutrient elements especially, Zn on plant growth may be due to its requirement in tryptophan synthesis (the precursor of IAA) and stimulation of IAA enzyme synthesis [Salisbury and Ross 1992] or its effect on improving growth hormone biosynthesis [Brady and Ray 2002]. Our results are in accordance with Abd El-Baky et al. [2010] who found that the combined application of higher K and Zn rates was more effective in growth promotion than individual application of either nutrient. Similar findings have been reported on various species [Potarzycki and Grzebisz 2009, 2012, Salim et al. 2014].

In contrast, the reduced photosynthetic function in untreated plants due to Zn and K deficiencies limited growth and assimilate translocation, and consequently dry weight [Hansch and Mendel 2009, Kanai et al. 2011].

Relative water content. The RWC of damask rose leaves increased significantly with increasing K or Zn level, more so when combined (Tab. 2). RWC is a suitable index of plant water status, and K is indispensable in the maintenance of cell turgor pressure, which is required for cell expansion [Rogalski 1994]. Furthermore, K has an important role in plant water

Table 1. Vegetative growth in damask rose plants after foliar applications of potassium (K) and/or zinc (Zn)

Treatments	Plant height (cm)	Number of main branches ($hill^{-1}$)	Number of secondary branches ($hill^{-1}$)	Leaf dry weight (%)	Stem dry weight (%)
T1	91.51 ±0.54g	22.20 ±0.38d	80.89 ±1.57e	18.56 ±0.27f	42.46 ±0.25e
T2	93.49 ±0.63e	23.57 ±0.35cd	81.19 ±0.69de	21.53 ±0.34e	42.99 ±0.10e
T3	97.83 ±0.82c	25.42 ±0.86c	82.56 ±0.64d	25.42 ±0.29c	44.60 ±0.23cd
T4	92.60 ±0.84fg	24.85 ±0.58c	89.20 ±0.85c	19.63 ±0.30d	42.70 ±0.31e
T5	95.56 ±0.78d	26.34 ±0.89bc	93.32 ±0.40b	19.79 ±0.10d	43.41 ±0.35de
T6	100.34 ±1.01b	27.55 ±0.92b	92.34 ±0.87b	24.54 ±0.32b	46.44 ±0.29b
T7	103.53 ±1.14a	30.18 ±0.56a	96.12 ±0.65a	28.54 ±0.28a	48.49 ±0.28a

Values in each column with different letters differ significantly from each other according to Duncan's multiple range test at P = 0.05 (n = 8). T1: control (water sprayed), T2: 0.5% K_2SO_4, T3: 1% K_2SO_4, T4: 0.5% $ZnSO_4$, T5: 1% $ZnSO_4$, T6: 0.5% K_2SO_4 + 0.5% $ZnSO_4$, and T7: 1% K_2SO_4 + 1% $ZnSO_4$

Table 2. Relative water content (RWC), stomatal conductance, and flower yield in damask rose plants after foliar applications of potassium (K) and/or zinc (Zn)

Treatments	RWC (%)	Stomatal conductance (mol H_2O m^{-2} s^{-1})	Total flower number ($hill^{-1}$)	Flower weight (g $hill^{-1}$)
T1	86.61 ±0.37e	0.17 ±0.012d	780.06 ±9.09f	1672 ±29.81f
T2	91.20 ±0.11c	0.27 ±0.005c	833.64 ±7.85d	1824 ±20.56d
T3	93.69 ±0.39b	0.28 ±0.022c	852.23 ±4.03c	1895 ±10.66c
T4	88.73 ±0.59d	0.22 ±0.005b	824.67 ±6.13e	1836 ±11.12e
T5	90.63 ±0.23c	0.23 ±0.017b	836.62 ±7.13d	1883 ±12.17d
T6	94.21 ±0.10ab	0.30 ±0.019a	897.71 ±6.13b	1925 ±15.72b
T7	95.95 ±0.14a	0.31 ±0.017a	922.00 ±6.68a	1971 ±27.85a

Values in each column with different letters differ significantly from each other according to Duncan's multiple range test at P = 0.05 (n = 8). T1: Control (water sprayed), T2: 0.5% K_2SO_4, T3: 1% K_2SO_4, T4: 0.5% $ZnSO_4$, T5: 1% $ZnSO_4$, T6: 0.5% K_2SO_4 + 0.5% $ZnSO_4$, and T7: 1% K_2SO_4 + 1% $ZnSO_4$

relations [Marschner 1995] by enhancing cell turgor through osmotic adjustment [Maathuis and Sanders 1996]. The RWC of two oilseed species increased significantly with K fertilization [Fanaei et al. 2009]. Similar responses to K application have been reported in maize [Zhang et al. 2014]. Increasing RWC due to Zn application could be due to the role of Zn in improving stomatal conductance, which was reflected in the maintenance of RWC [Arough et al. 2013].

Stomatal conductance. Stomatal conductance increased significantly in damask rose leaves supplied with either foliar K or Zn fertilizer, relative to the control, but no differences were observed between the two application rates. The K treatments increased stomatal conductance more than the Zn treatments. The combined K and Zn treatments resulted in the maximum stomatal conductance (Tab. 2). Potassium plays an important role in stomatal function by maintaining turgor pressure [Pervez et al. 2004]. The results of this study agree with those of Tsialtas et al. [2016] who reported that foliar K application enhanced leaf gas exchange, sustained open stomata, and increased transpiration rate, thereby increasing stomatal conductance.

These findings support previous reports by Zhao et al. [2001] and Pervez et al. [2004] on cotton, where K had a positive effect on stomatal conductance. Increased stomatal conductance in other species in response to foliar application of Zn has also been documented on chickpea [Khan et al. 2004] and maize [Wang et al. 2009].

Total flower number and weight/hill. The individual K or Zn treatments significantly increased flower number and flower weight per hill, relative to the control, more so at the higher application rates (Tab. 2). The combined Zn and K treatment at the higher level (T7) produced the most flowers (922) and flower weight (1971 g) per hill, which equated to respective increases of 18.20% and 17.88%, relative to the control.

The increased flower yield may be due to the role of K in increasing branch number and hence flower number per hill. These results support those of Kumar et al. [2016b] who found that K application significantly increased flower number and flower yield per plant in damask rose. Potassium activates several enzymes, including those involved in carbohydrate synthesis, and is involved in organic acid neutralization and cell division promotion [Ruiz 2006]. Foliar K application is required to supplement soil nutrients for maximizing yield [Waraich et al. 2011]. Similar findings to current study have been reported by Finnan and Burke [2013] and Hu et al. [2016].

Zinc plays a crucial role in plant growth and yield of several aromatic and medicinal plants [Abd El-Wahab 2008]. The increased flower yield in response to Zn application in our study agrees with Kumar et al. [2016a] who demonstrated that foliar application of Zn increased damask rose flower yield per plant, relative to the control. A similar finding was reported for *Matricaria chamomilla* [Nasiri et al. 2010, Nasiri and Najafi 2015]. Khalifa et al. [2011] reported a significant increase in flower characteristics and yield per plant in iris with Zn foliar spraying. In contrast, foliar Zn application did not affect the yield of savory (*Satureja hortensis*) [Shiriyan et al. 2014].

Table 3. Volatile oil percentage and yield in damask rose plants after foliar applications of potassium (K) and/or zinc (Zn)

Treatments	Volatile oil (%)	Volatile oil yield (L ha^{-1})
T1	0.030 ±0.003e	1254 ±0.058g
T2	0.032 ±0.004d	1459 ±0.085f
T3	0.035 ±0.005bc	1658 ±0.0114c
T4	0.034 ±0.005c	1561 ±0.0139e
T5	0.036 ±0.005b	1695 ±0.073d
T6	0.041 ±0.005a	1973 ±0.069b
T7	0.043 ±0.005a	2119 ±0.0104a

Values in each column with different letters differ significantly from each other according to Duncan's multiple range test at P = 0.05 (n = 8)
T1: control (water sprayed), T2: 0.5% K_2SO_4, T3: 1% K_2SO_4, T4: 0.5% $ZnSO_4$, T5: 1% $ZnSO_4$, T6: 0.5% K_2SO_4 + 0.5% $ZnSO_4$, and T7: 1% K_2SO_4 + 1% $ZnSO_4$

Volatile oil content and composition. The volatile oil content percentage and volatile oil yield increased significantly with individual foliar applications of K or Zn, relative to control, more so at the higher rate (Tab. 3). The combined applications of K and Zn produced significantly higher volatile oil contents than the individual applications of K or Zn. The combined treatment at the higher level (T7) produced the maximum volatile oil content; the volatile oil yield per ha was 68.99% higher than the control.

The increase in volatile oil content in this experiment due to K supply supports the findings of Mollafilabi et al. [2010] on *Mentha piperita* L. and Khalid [2013] on *Calendula officinalis* L. Zinc foliar application increased volatile oil content, relative to the control, in mint [Akhtar et al. 2009], coriander [Said-Al Ahl and Omer 2009], chamomile [Nasiri et al. 2010], and thyme [Yadegari 2012]. However, Zn supply did not affect volatile oil percentage in savory [Shiriyan et al. 2014] or damask rose [Kumar et al. 2016a].

The GC-MS analysis of the volatile oil from damask rose leaves identified 49 compounds (Tab. 4), with the main components being linalool (6.88–7.62%), nerol (6.80–7.47%), citronellol (18.49–19.49%), geraniol (14.84–15.46%), and nonadecane (6.42–6.92%). All treatments produced a similar profile, but there were quantitative differences. Individual foliar applications of K or Zn affected the percentages of the volatile oil components, which generally increased relative to the control. The combined treatments recorded the highest values, particularly at the higher application rate (Tab. 4). In the T7 treatment, rose leaves contained 21.69 and 16.96% of citronellol and geraniol, compared with 19.80 and 15.80% in the control, respectively. These findings agree with those of Kürkçüoglu et al. [2013], Abdel-Hameed et al. [2016] and Kumar et al. [2016a] on damask rose oil. Changes in the percentages of volatile oil components due to K application may be due to the effects of K on metabolism and enzyme activity through the metabolic pathways of essential oil [Marschner 1995]. Volatile oil composition decreased, increased or remained unaffected with K application in several aromatic plants [Mollafilabi et al. 2010]. Similar effects of K on the chemical constituents of volatile oils have been reported [Khalid et al. 2007, Khalid 2013]. Kumar et al. [2016a] also reported quantitative and qualitative differences in damask rose volatile oil in response to Zn application. Likewise, the volatile oil composition of several aromatic plants was influenced by Zn supply [Said-Al Ahl and Omer 2009, Yadegari and Shakerian 2014].

Nutrient elements. Individual foliar applications of K or Zn significantly increased N, P, K, and Zn contents in damask rose leaves, relative to the control (Tab. 5). The K treatment enhanced the percentages of N, P, and K, relative to the Zn treatment, while the Zn treatment increased the percentage of Zn, relative to the K treatment. Higher percentages were recorded when higher levels of K or Zn were applied. The highest values occurred in the combined treatment with K and Zn at the higher level (T7).

Potassium is an essential element that plays a crucial role in transport and uptake processes, osmoreg-

Table 4. Volatile oil constituents in damask rose plants after foliar applications of potassium (K) and/or zinc (Zn)

No.	RI	Compound	Contents (%)						
			T1	T2	T3	T4	T5	T6	T7
1	1025	α-Pinene	3.70	3.80	3.70	3.90	3.90	4.02	4.06
2	1124	Sabinene	0.10	0.10	0.11	0.11	0.12	0.10	0.10
3	1138	β-Pinene	0.70	0.70	0.60	0.50	0.60	0.70	0.60
4	1177	Myrcene	1.90	1.88	1.92	1.93	1.91	1.94	1.95
5	1207	Limonene	0.30	0.31	0.30	0.29	0.31	0.32	0.28
6	1218	1,8-Cineole	0.20	0.21	0.19	0.18	0.17	0.21	0.20
7	1252	p-Cymene	0.20	0.21	0.20	0.19	0.23	0.21	0.27
8	1549	Linalool	6.88	7.13	7.10	7.17	7.22	7.49	7.62
9	1558	*cis*-Rose oxide	0.70	0.71	0.76	0.69	0.71	0.81	0.87
10	1560	Phenyl ethyl alcohol	2.60	2.58	2.63	2.61	2.58	2.65	2.73
11	1569	*trans*-Rose oxide	0.60	0.61	0.61	0.62	0.63	0.67	0.66
12	1582	Calarene (= β-gurjunene)	0.10	0.10	0.11	0.12	0.11	0.10	0.13
13	1584	Terpinen-4-ol	1.20	1.25	1.23	1.22	1.24	1.29	1.28
14	1590	α-Terpineol	2.53	2.48	2.59	2.55	2.51	2.61	2.63
15	1603	Nerol	6.80	6.98	7.34	7.04	7.12	7.26	7.47
16	1617	Citronellyl formate	0.20	0.19	0.24	0.21	0.22	0.24	0.23
17	1655	Neryl formate	0.10	0.11	0.12	0.09	0.10	0.12	0.14
18	1667	Neral	0.60	0.62	0.73	0.64	0.61	0.67	0.71
19	1672	Heptadecane	1.40	1.42	1.48	1.41	1.45	1.52	1.49
20	1684	Geranyl formate	0.30	0.28	0.38	0.31	0.34	0.37	0.38
21	1691	1-Heptadecene	0.40	0.41	0.43	0.41	0.40	0.45	0.44
22	1702	Citronellol	18.49	18.58	18.69	18.78	18.89	19.18	19.49
23	1727	Geraniol	14.84	14.92	15.01	14.86	15.08	15.16	15.46
24	1735	Geranial	1.20	1.23	2.79	2.61	2.36	2.56	2.47
25	1739	Citronellyl acetate	0.72	0.75	0.85	0.74	0.79	0.82	0.86
26	1744	Eugenol	1.62	1.70	1.81	1.65	1.69	1.74	1.82
27	1748	Geranyl acetate	0.91	0.92	0.96	0.84	0.95	0.98	0.97
28	1756	Methyl eugenol	1.34	1.35	1.37	1.39	1.40	1.44	1.48
29	1765	β-Caryophyllene	0.90	0.93	0.98	0.91	0.92	0.96	0.98
30	1772	α-Guaiene	1.23	1.22	1.25	1.28	1.33	1.37	1.41
31	1788	α-Humulene	0.70	0.72	0.76	0.72	0.71	0.82	0.84
32	1794	Germacrene D	0.62	0.64	0.73	0.63	0.65	0.72	0.70
33	1802	δ-Guaiene	1.08	1.06	1.09	1.02	1.04	1.11	1.15
34	1807	Pentadecane	0.52	0.56	0.64	0.53	0.54	0.62	0.68
35	1811	Caryophyllene oxide	0.43	0.48	0.49	0.42	0.45	0.52	0.50
36	1817	Octadecane	0.31	0.32	0.42	0.34	0.37	0.47	0.51
37	1820	Nonadecene	2.65	2.79	2.68	2.65	2.69	2.89	3.05
38	1831	Nonadecane	6.42	6.59	6.67	6.67	6.58	6.81	6.92
39	1843	1-Eicosane	0.45	0.48	0.46	0.45	0.44	0.57	0.52
40	1867	(E)-Nerolidol	0.40	0.41	0.51	0.47	0.48	0.47	0.53
41	2057	Heneicosane	1.02	1.14	1.07	1.02	1.06	1.17	1.23
42	2102	Heneicosene	0.21	0.24	0.21	0.20	0.22	0.24	0.25
43	2115	(E)-3,7-Dimethyl-5-octen-1,7-diol	0.23	0.22	0.27	0.21	0.25	0.25	0.26
44	2192	α-Cadinol	0.13	0.15	0.13	0.12	0.13	0.12	0.16
45	2244	Tricosane	0.30	0.32	0.29	0.30	0.31	0.34	0.41
46	2267	(2E,6Z)-Farnesol	0.24	0.25	0.23	0.22	0.26	0.25	0.28
47	2284	(2E, 5E)-3,7-Dimethyl-2,5-octadien-1,7-diol	0.12	0.15	0.14	0.13	0.12	0.13	0.14
48	2309	(Z)-9-Tricosene	0.10	0.10	0.13	0.13	0.12	0.14	0.15
49	2352	Geranic acid	0.11	0.10	0.13	0.10	0.11	0.12	0.15
		Total	**88.8**	**90.4**	**93.53**	**91.58**	**92.42**	**95.72**	**97.61**

RI: retention indices, T1: control (water sprayed), T2: 0.5% K_2SO_4, T3: 1% K_2SO_4, T4: 0.5% $ZnSO_4$, T5: 1% $ZnSO_4$, T6: 0.5% K_2SO_4 + 0.5% $ZnSO_4$, and T7: 1% K_2SO_4 + 1% $ZnSO_4$

Table 5. N, P, K, and Zn contents in damask rose leaves after foliar applications of potassium (K) and/or zinc (Zn)

Treatments	N (%)	P (%)	K (%)	Zn (mg L^{-1})
T1	2.08 ±0.07f	0.34 ±0.02d	2.11 ±0.05e	33 ±2.43f
T2	2.39 ±0.22e	0.40 ±0.01c	2.19 ±0.02d	38±1.26e
T3	2.60 ±0.27c	0.42 ±0.01b	2.33 ±0.05b	39 ±0.48e
T4	2.46 ±0.09d	0.38 ±0.01c	2.24 ±0.04c	58 ±0.94d
T5	2.54 ±0.07c	0.41±0.01b	2.33 ±0.02b	66 ±1.63c
T6	2.76 ±0.09b	0.45 ±0.02a	2.40 ±0.02a	70 ±1.25b
T7	2.85 ±0.12a	0.48 ±0.01a	2.44 ±0.03a	73 ±1.25a

Values in each column with different letters differ significantly from each other according to Duncan's multiple range test at P = 0.05 (n = 8)
T1: control (water sprayed), T2: 0.5% K_2SO_4, T3: 1% K_2SO_4, T4: 0.5% $ZnSO_4$, T5: 1% $ZnSO_4$, T6: 0.5% K_2SO_4 + 0.5% $ZnSO_4$, and T7: 1% K_2SO_4 + 1% $ZnSO_4$

ulation, and enzyme activation [Marschner 1995]. In accordance with our findings, Hu et al. [2016] reported a linear response of N to K supply, supporting the hypothesis that N metabolism is affected by K supply. The increase in K percentage may also be due to increased nutrient availability [Marschner 1995]. Similar results have been reported elsewhere [Yu et al. 1996, Kanai et al. 2011, Salim et al. 2014]. Oddly, Tsialtas et al. [2016] found that K supply did not affect K leaf content and they surmised that the absorbed K was stored in other plant parts; perhaps there was a dilution of K in the biomass yield [Pettigrew 2003]. Similar to the K effect, foliar application of Zn increased N, P, K and Zn contents in damask rose leaves, which agrees with the findings of Khalifa et al. [2011]. Havlin et al. [1999] credited the increased nutrient levels in leaves to the role of Zn in sugar regulation and the enzymes that control plant growth. The increased Zn percentage in rose leaves may be attributed to the availability of Zn sprayed on leaves [Said-Al Ahl and Omar 2009]. The combined application of Zn and K produced the greatest increases in N, P, K and Zn content in leaves, which is supported by others [Abd El-Baky et al. 2010, Sarrwy et al. 2012, Dimkpa et al. 2017].

Chlorophyll content. Chlorophyll content increased with increasing K or Zn levels (Fig. 1A). Foliar application of Zn produced more chlorophyll than K. The combined K and Zn treatments produced more chlorophyll than the individual treatments, with the T7 treatment producing the most (1.36 mg g^{-1} FW). Chlorophyll content is negatively affected by nutrient deficiencies, which reduces photosynthetic function [Younis et al. 2013]. The photosynthetic apparatus is directly influenced by the biosynthesis and functioning of key photosynthetic components [Kalaji et al. 2014]. Potassium deficiency reduced the leaf net photosynthetic rate, photosynthetic phosphorylation activity, and electron transfer energy in cotton plants [Zhao et al. 2001]. Potassium deficiency is considered the main cause of reduced chlorophyll content and photosynthesis due to stomatal conductance restrictions [Reddy and Zhao 2005, Pettigrew and Gerik 2007]. The improvements in chlorophyll content may be due to the function of K in biochemical pathways, which increases the photosynthetic rate and CO_2 assimilation, and facilitates carbon movement [Sangakkara et al. 2000]. A similar effect of K on chlorophyll content has been reported in other species [Kanai et al. 2011, Younis et al. 2013, Hu et al. 2016, Zhao et al. 2016]. The promotion effect of Zn on chlorophyll content may be attributed to its role as a component of carbonic anhydrase and various dehydrogenases, and in auxin production and CO_2 assimilation [Said-Al Ahl and Omer 2009]. Our results agree with those of Khalifa et al. [2011] who found that pigment content in iris plants significantly increased with Zn foliar spraying. Moreover, Potarzycki and Grzebisz [2009] showed that Zn enhanced photosynthesis, chlorophyll synthesis, and carbon anhydrase activity in maize. Furthermore, Zn can modulate plant chlorophyll content in their own right [e.g., Dimkpa et al. 2018, 2019, 2020, Taran et al. 2017].

Total soluble sugars. Foliar application of K or Zn significantly increased TSS content in damask rose leaves, relative to the control, more so with K application. The combined treatments of K and Zn (T6 and

T7) recorded the highest TSS contents, more so in T7 (Fig. 1B). These increases in TSS are likely due to the role of K in carbohydrate metabolism [Hu et al. 2015]. In addition, K is essential for motivating plasmalemma ATPase that produces the necessary conditions for metabolites, such as sucrose [Barker and Pilbeam 2007]. Potassium also stimulates starch synthase enzyme activity associated with starch synthesis [Mengel and Kirkby 1987]. Yadav et al. [2014] reported that K foliar application improved TSS content in *Ziziphus mauritiana*. Our results are in accordance with Sarrwy et al. [2012] who revealed that TSS increased significantly with foliar K application.

Zinc is essential for sugar and enzyme regulation, which is reflected in increasing TSS [Havlin et al. 1999]. Our results support the findings of Khalifa et al. [2011] who reported a significant improvement in TSS with Zn supply. Similarly, Sarrwy et al. [2012] revealed that TSS in mandarin leaves improved significantly with foliar application of K and Zn.

Free amino acid and soluble protein contents. Free amino acid content declined significantly with K

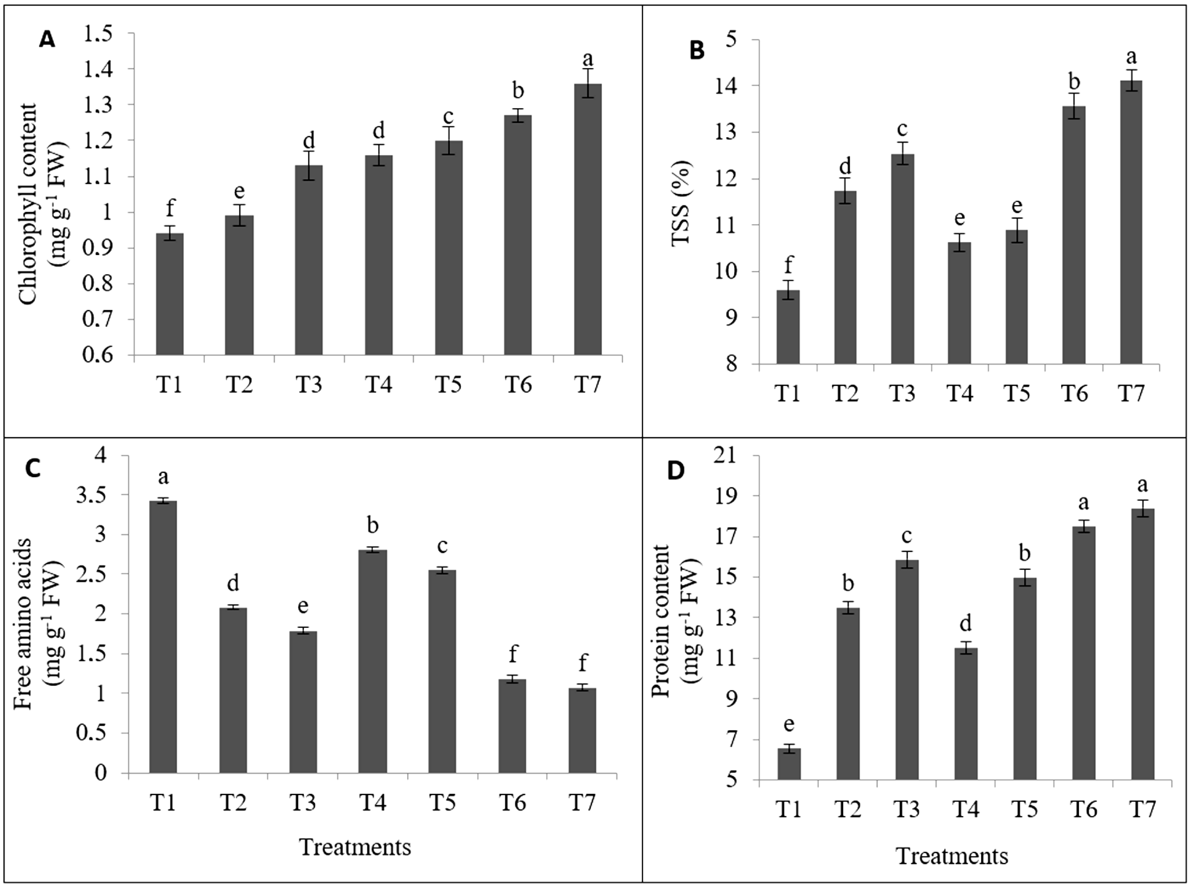

Fig. 1. (A) Chlorophyll content, (B) total soluble sugars (TSS), (C) free amino acids, and (D) protein content in damask rose plants after foliar applications of potassium (K) and/or zinc (Zn). Columns with different letters differ significantly from each other according to Duncan's multiple range test at $P = 0.05$ ($n = 8$). T1: control (water sprayed), T2: 0.5% K_2SO_4, T3: 1% K_2SO_4, T4: 0.5% $ZnSO_4$, T5: 1% $ZnSO_4$, T6: 0.5% K_2SO_4 + 0.5% $ZnSO_4$, and T7: 1% K_2SO_4 + 1% $ZnSO_4$

or Zn foliar application, relative to the control (Fig. 1C). The higher K level treatment resulted in a gradual and significant reduction in free amino acid content, but this was not the case for Zn. The combined treatments with K and Zn recorded the lowest content of free amino acids, which declined by 31.19–34.40%, relative to the control.

In contrast, protein content increased significantly with K or Zn application, more so at the higher rate. The combined treatment at the higher rate (T7) recorded the highest protein contents (Fig. 1D). Potassium plays an important role in the processes of osmoregulation, protein synthesis, phloem loading, and transport and uptake [Marschner 1995]. Amino acids and proteins as nitrogenous compounds are the main products of NO_3 assimilation [Causin 1996]. Potassium application improved N metabolism, and hence increased synthesis of amino acids [Ruan et al. 1998]. In this study, increased leaf N content suggests that nitrogen metabolism also improved with K application; however, free amino acid content declined in the K treatments due to a reduced amino acid export rate in phloem under K deficiency [Wang et al. 2012]. This is a possible explanation for the reduction in growth characters and yield under K deficiency (T1) (Tables 1 and 2). Ruiz and Romero [2002], Salim et al. [2014] and Hu et al. [2016] reported similar reductions in free amino acid content and increases in protein content as a result of K application. Our results suggest that the distribution of nitrogenous compounds (between amino acids and protein) and their transformation changed due to K supply. Hu et al. [2016] credited the decline in free amino acid content and increase in protein content to increases in nitrate reductase, glutamine synthetase, and glutamate synthase and reductions in protease and peptidase enzyme activities under adequate-K supply.

Zinc is required as a structural and catalytic component of proteins and enzymes for normal growth and development [Broadley et al. 2007]. Zinc is also involved in physiological processes including protein synthesis [Cakmak 2000, Potarzycki and Grzebisz 2009]. Since Zn affects N assimilation (Tab. 6), an increase in protein content and decrease in free amino acid content were expected. These results are in accordance with those of Hisamitsu et al. [2001] who observed a significant increase in protein content in maize with Zn application.

CONCLUSION

Growth, flower yield and volatile oil content in damask rose increased with K or Zn foliar application, in this regard, the greatest improvements occurred in the combined treatments (T6 and T7). In the same direction, relative water content, stomatal conductance, chlorophyll content, total soluble sugars, and protein content were significantly increased. At the same time, free amino acid content declined, suggesting that K and Zn supply influenced the distribution of amino acids and proteins. Moreover, N, P, K, and Zn contents in damask rose leaves increased, relative to the control. The positive effects of K or Zn were more pronounced with combined application, the higher level (T7) was a superior in this regard, relative to the individual applications. Foliar application of K and Zn should provide damask rose plants with the required nutrition to increase the quantity and quality of flowers and volatile oil yields.

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