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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

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Abstract: Iron (Fe) deficiency exists as a widespread nutritional disorder in alkaline and calcareous soils; therefore, Fe-enriching strategies may be used to overcome this issue. Field experiments were conducted with a randomized complete design with three replicates for evaluating the effectiveness of iron oxide nanoparticles (Fe-NPs) against traditional Fe compounds (sulfate or chelate), which have various shortcomings on Rose-scented geranium (RSG) herb in terms of plant growth, phytopharmaceuticals, essential oil (EO), and its constituents. Supplementation of Fe-sources considerably improved RSG plant growth and EO yield in the 1st and 2nd cut throughout the two seasons over non-treated control plants. A total of 11 compounds of RSG-EO were identified; the main constituents were citronellol, geraniol, and eugenol. The results indicate that EO composition was significantly affected by Fe-sources. Amendments of Fe-sources considerably augmented photosynthetic pigments, total carbohydrates, nitrogen, phosphorous, potassium, iron, manganese, zinc, phenols, flavonoids, and anthocyanin. Commonly, Fe-NPs with humic acid (Fe-NPs-HA) supplementation was superior to that of traditional sources. The highest values were recorded with spraying Fe-NPs-HA at 10 mg L⁻¹ followed by 5 mg L⁻¹, meanwhile, the lowest values were recorded in untreated control plants. Current findings support the effectiveness of nanoparticle treatment over Fe-sources for improving growth and yield while also being environmentally preferred in alkaline soil. These modifications possibly will be applicable to EO quality and its utilization in definite food and in medical applications.

Keywords: chlorophyll; essential oil; nano-iron; phytopharmaceutical; rose-scented geranium; yield

1. Introduction

Rose-scented geranium (RSG, *Pelargonium graveolens* L. Her. ex Ait. 'Synonym *Prasophyllum roseum* Willd.'; Geraniaceae) is a highly valued perennial aromatic shrub worldwide [1]. The chief RSG production takes place in China and the Middle East, i.e., Egypt [2]. Its EO is extensively used in the perfumery, cosmetic, and aromatherapy industries [1,3,4]. Additionally, they are becoming increasingly popular for several human disorders, i.e., relieving dysentery, cancer, sterility, urinary stones, and liver complications [5,6]. The main constituents of RSG-EO are citronellol (19.28–40.23%), geraniol (6.45–18.40%), linalool (3.96–12.90%), iso-menthone (5.20–7.20%), citronellyl formate (1.92–7.55%), Guaia-6,9-diene

(0.15–4.40%), and bits of more than 100 constituents [1,4]. Accordingly, EO composition is strongly affected by environmental factors and micronutrients including iron [7,8].

Iron (Fe) represents the 4th supreme plentiful element in the earth's crust, which participates in several species' physio-biochemical pathways [9,10]. It is a co-factor for approximately 140 enzymes elaborated in photosynthesis, gas exchange, nitrogen fixation, and nucleic acid assimilation [11,12]. It is also involved in chlorophyll biosynthesis, chloroplast development, and electron transport systems [13,14]. Iron deficiency (FDS) is a widespread threat affecting 30–50% of cultivated alkaline soils in dry regions, i.e., Egyptian soil [15,16]. Considering the soil–plant–animal–human food chain FDS not only affects plant growth and development but can also accelerate anemia in animals and humans [1]. Therefore, usage of the proper amount and forms of Fe is a prerequisite to extra studies, to lessen FDS, and to increase nutrient-use efficiency. Presently, several products were applied to overcome FDS. The EU Directive No. 2003/2003 [17,18] comprises chelates i.e., ethylene diaminetetraacetic acid (Fe-EDTA) and ethylenediamine-*N, N'*-bis(hydroxyphenyl) acetic acid (Fe-o,o-EDDHA) complexes; and inorganic salts as a promising method for improving Fe uptake and lessens Fe-chlorosis. The effectiveness of inorganic and chelated Fe fertilizers in mitigating FDS is exceedingly variable depending on their solubility, constancy, infiltration capacity via leaf cuticle and translocation into the plant tissues [19,20]. The usage of Fe chelates does not represent a viable approach for agronomists to avoid Fe chlorosis as a result of the excessive cost and ecological hazards [21]. Furthermore, most of these chelates are recalcitrant products in soils and waters, and there has been developing anxiety recently about the ecological threat of their amendment to soils [22].

Recently, there has been a thrust to develop innovative nanoparticle (NP) fertilizer formulations including iron nano-oxide (Fe-NPs), for reducing the quantity of conventional fertilizers owing to (1) their unique physical and chemical attributes (small size, huge surface area, pureness, and steadiness), and (2) the interface amongst nanoparticles and biomolecules possibly will provoke metabolic pathways in treated plants [8,18,23]. The stimulating impact of Fe-NPs on the growth and economic yield of different herbs has been reported previously [8,23,24]. In this regard, El-Khateeb et al. [8] on sweet marjoram found that Fe-NPs foliar spraying augmented plant growth, chlorophyll concentration, carbohydrates, EO %, and yield as well as their constituents. Nejad et al. [25] found that Fe-spraying increased the photosynthetic pigments, phenols, and EO % of RSG plants. Gutierrez-Ruelas et al. [18] recorded that Fe-NPs spraying increased green bean plant biomass, total chlorophyll, and Fe content as well as nitrate reductase activity.

However, it is unclear how Fe-NP supplementation affects RSG plant development and some biochemical characteristics when used in place of conventional Fe-sources. As a result, the main goal of the current study is to determine the effects of Fe-sources (chelate, sulfur, and nano) on the growth of RSG, EO content, and their constituents, as well as their phytochemicals. We hypothesized that various Fe sources have varying effects on plant growth, EO yield, and composition as well as phytopharmaceuticals production. As a novel Fe source, Fe-NPs were also very successful in eliciting the accumulation of phytopharmaceuticals, as well as boosting EO yield and plant antioxidant activity.

2. Materials and Methods

2.1. Assimilation of Fe-NPs

The synthesis of magnetic iron oxide nanoparticles (Fe-NPs) was created with an eco-friendly adapted scheme [26]. The co-precipitation method synthesized the Fe-NPs in situ, which is a classical method for Fe₃O₄ generation. Concisely, 6.1 g of ferric chloride was dissolved in 100 mL of distilled water, subsequently, the addition of an aliquot of concentrated HCl to evade Fe(OH)₃ precipitation, afterward 4.2 g of FeSO₄·7H₂O were dissolved in a mix, and heated to 90 °C, then 10 mL of NH₄OH (25%) was poured quickly, and pH of the solution was sustained at 10. The mixture was stirred at 90 °C for 30 min and then cooled to lab temperature. The black substance was collected via centrifugation at 600× *g*, and then washed with ethanol and distilled water.

2.2. Characterization of Fe-NPs

The dimension and shape of Fe-NPs were detected by transmission electron microscopy (TEM, JEOL Ltd. Tokyo, Japan). The TEM samples were prepared via dropping solution on a carbon-coated copper grid and then exposed to the infra-light for 30 min (Okenshoji Co., Ltd., Tokyo, Japan microgrid B). The micrograph was examined by JEOL-JEM 6510 at 70 kV in the RCMB, Mansoura University, Egypt.

2.3. Experimental Location, Climate Data, and Soil Properties

Two field experiments were done at a private farm in El-Serw City (31°14'19.21" N, 31°39'13.64" E; 16 m ASL), Damietta, Egypt, during the 2018 and 2019 seasons for assessing the response of RSG plant growth, yield, and EO content to foliar applications of Fe-sources. Physical-chemical examination of the soil surface (0–60 cm) was employed before transplanting [27]. The soil texture was clay, and its properties were recorded in Table 1. Diurnal experimental site ecological information involved temperature, solar radiation, relative humidity, and wind speed of the 1st and 2nd seasons as presented in Supplementary Materials Table S1.

Table 1. Physical and chemical analyses of the experimental soil in two seasons.

Soil Properties		Values	
		1st Season	2nd Season
Particle size distribution (%)	Sand (%)	21.00	21.19
	Silt (%)	35.92	34.82
	Clay (%)	43.08	44.08
Some physical and chemical trials	Electrical conductivity (dSm ⁻¹)	4.070	4.060
	pH (soil paste)	7.630	7.700
	Calcium carbonate (%)	3.730	3.810
	Nitrogen (mg kg ⁻¹ soil)	20.32	21.03
	Phosphorus (mg kg ⁻¹ soil)	16.72	17.63
Cations (meq 100 g ⁻¹ soil)	Calcium	2.000	4.000
	Magnesium	11.33	12.12
	Sodium	2.740	2.720
	potassium	2.060	2.090
Anions (meq 100 g ⁻¹ soil)	Carbonate	0.000	0.000
	Bicarbonate	0.370	0.360
	Chloride	4.690	4.650
	sulfate	5.630	5.690

2.4. Experimental Layout

The experimental soil was mechanically plowed twice prior to transplantation until the soil surface was steady and established in the plots. Uniform seedlings of 25–30 cm length (from the Dept. of Medicinal and Aromatic Plants, Ministry of Agric., Egypt) were individually transplanted on 1st March, during the 2018 and 2019 seasons, in 3 × 3.5 m plots, rows with 60 cm apart and 60 cm amongst the seedlings. In both seasons, the plants were received the recommended doses of mineral fertilizers (ammonium sulfate '20.5%', calcium superphosphate '15.5%', and potassium sulfate '52%' at 200, 100, and 55 kg/fed. '4200 m²', correspondingly) before planting and once first cut in both seasons. Entirely agricultural practices of plants were carried out following the endorsements of the Ministry of Agriculture, Egypt. The design of the experiment was completely randomized that contained 11 treatments at three replicates, and they are displayed in Table 2. The

preliminary study provided the basis for choosing this concentration. The Fe-forms were sprayed directly on the plants four times at 45 and 60 days (for the 1st cut), and 135 and 150 days (for the 2nd cut) from transplanting (15 days prior to flowering and at the start of the flowering stage in both cuts).

Table 2. The experimental treatments and their identifications.

No.	Treatments	Abbreviation
1	Control (Spraying with tap water)	T1
2	Spraying with 5 mg L ⁻¹ Fe-NPs	T2
3	Spraying with 10 mg L ⁻¹ Fe-NPs	T3
4	Spraying with 5 mg L ⁻¹ Fe-NPs with humic (Fe-NPs-HA)	T4
5	Spraying with 10 mg L ⁻¹ Fe-NPs with humic (Fe-NPs-HA)	T5
6	Spraying with 100 mg L ⁻¹ ferric sulfate	T6
7	Spraying with 200 mg L ⁻¹ ferric sulfate	T7
8	Spraying with 100 mg L ⁻¹ EDDHA	T8
9	Spraying with 200 mg L ⁻¹ EDDHA	T9
10	Spraying with 100 mg L ⁻¹ EDTA	T10
11	Spraying with 200 mg L ⁻¹ EDTA	T11

2.5. Measurements and Data Collection

Plants were harvested (cuts) 10 cm above the soil two times at full bloom (after 90 and 180 days from transplanting) in each season for determining growth characteristics (plant height 'cm', branches number/plant, shoot fresh and dry weights 'g/plant') and EO (%), yield/plant, yield/fed.), meanwhile both cuts in the second season was used for determining photosynthetic pigments, ions, phytopharmaceuticals, and EO composition.

2.6. Determination of Essential Oil

Using a modified Clevenger apparatus for three hours, the EO was hydro-distilled from the air-dried plants that had been in the shade for 48 h [28]. After distillation, the EO was dried by a glass separator, filtered two times, kept in the fridge at 4 °C, and preserved in dark closed bottles for preventing light and oxygen exposure. EO % = (EO volume/shoot fresh weight) × 100. The EO yield (mL/plant) was calculated following the current equation; EO yield = shoot fresh weight (g) × EO%.

The EO components were recognized, with a Varian Chrompack CP-3800 gas chromatograph (Varian Company, California, USA) with a mass detector (4000 GC-MS/MS). Helium served as the gas carrier at a flow rate of 2 mL min⁻¹ with a linear velocity of 32 cm s⁻¹. The flame ionization detector temperature was 265 °C and the injector temperature was 250 °C. Detection of the constituents was dependent on a judgment of their mass spectra with those of a computer library or with realistic composites and validation of compound individualities was also gained via Retention index (RI) assessed regarding a homologous series of C5–C24 (n-alkanes) as designated by Adams [29].

2.7. Ion Concentration

Nitrogen (N), and phosphorus (P) were extracted and estimated [30] from the plant dry shoot. Roughly 0.2 g shoot dry mass was cautiously moved to a digestion flask with 5 mL of concentrated H₂SO₄, at 100 °C for 2 h; then, the combinations were cool for 15 min in lab temperature. An aliquot of H₂SO₄/HClO₃ mix was poured dropwise. Total N was assessed with the micro-Kjeldahl scheme. The outline of Cooper [31] was followed for the assessment of P alongside the phosphate standard curve. In the meantime, the potassium (K), Fe, manganese (Mn), and zinc (Zn) were extracted by acid digestion (70% nitric acid

and 35% hydrochloric acid) in a Milestone MLA 1200 Mega microwave digestion device, then estimated using iCAPTM 7000 Plus Series ICP-OES (Thermo ScientificTM, Boston, MA, USA, Boston) following Bettinelli et al. [32] protocol.

2.8. Photosynthetic Pigment

Chlorophylls and carotenoids were assessed by Lichtenthaler and Wellburn [33] procedure. Generally, 0.2 g FW from the 4th upper leaves was extracted overnight in pre-cooled methanol (96%) accompanied by 0.05% sodium bicarbonate. The optical density (OD) was read at 470, 653, and 666 nm spectrophotometrically (T60 UV-Visible spectrophotometer, Leicestershire, UK). Pheophytin (Pheo) and Chlorophyllide (Chlide) were assessed in the 4th upper leaves according to Radojevic and Bashkin [34] and Harpaz-Saad et al. [35], respectively. On the other hand, the protocol described by Sarropoulou et al. [36] was applied for the estimation of protoporphyrin (Proto), Mg-protoporphyrin (Mg-Proto), and protochlorophyllide (Pchlide).

2.9. Total Carbohydrates

The colorimetric technique designated by Zhang et al. [37] was used to estimate total carbohydrate concentrations in plant shoots using 3, 5-dinitrosalicylic acid (DNS), after extraction with hot ethanol (80%). An aliquot of shoot extract (3 mL) was mixed with 3 mL DNS reagent in a test tube, then heated in a boiling water bath for 5 min. Consequently, 40% Rochelle salt solution (1 mL) was quickly added, to the mix, and placed in a water bath at lab temperature for about 25 min., subsequently; the OD at 510 nm is recorded with a spectrophotometer (T60 UV-Visible spectrophotometer, Leicestershire, UK).

2.10. Total Phenolic Compounds, Total Flavonoids, and Total Anthocyanin

The Folin–Ciocalteu procedure was utilized spectrophotometrically (T60 UV-Visible spectrophotometer, Leicestershire, UK) to estimate the total phenolic concentration [38]. Concisely, the ethanolic plant extract was added to the Folin–Ciocalteu reagent and sodium carbonate solution (20%), homogenized, and incubated in the dark for 30 min. The OD was then measured at 650 nm. A calibration curve for gallic acid was used to estimate their concentration (mg gallic g⁻¹ DW).

The technique established by Meda et al. [38] was employed to assess the total concentration of flavonoids (mg quercetin g⁻¹ DW) using the aluminum chloride colorimetric scheme. An aliquot of ethanolic extract, 0.1 mL of aluminum chloride, 0.1 mL of sodium acetate, and 2.8 mL of distilled water was combined and stirred. The mixture's OD was deliberate spectrophotometrically (T60 UV-Visible spectrophotometer, Leicestershire, UK) at a wavelength of 415 nm.

Total anthocyanin concentration was determined according to the method of Abdel-Aal and Hucl [39], in which the OD of each pre-chilled acidified methanolic extract was assessed spectrophotometrically (T60 UV-Visible spectrophotometer, UK) at 530 nm. The concentration (mg 100 g⁻¹ FW) was expressed as cyaniding-3-glucoside using a molar extinction coefficient of 27.900.

2.11. Statistical Analysis

The similarity of variables error variance was performed earlier in the analysis of variance (ANOVA). The outputs demonstrated that all data satisfied the uniformity to accomplish further ANOVA checks. The data acquired were exposed to one way-ANOVA at a 95% confidence level by CoHort Software, 2008 statistical package (CoHort software, 2006; Raleigh, NC, USA). The mean values of treatments were compared via Tukey's HSD-MRT test at $p \leq 0.05$. Values attended by diverse letters were significantly different at $p \leq 0.05$. The data presented are mean values \pm standard error (SE). The levels of significance were denoted by * $p < 0.05$, ** at $p < 0.01$, *** $p < 0.001$ and NS, no significant.

Table 3. Cont.

Treatments	Second season							
	Cut 1				Cut 2			
	Plant Height (cm)	Branches No/Plant	Shoot fresh weight (g)	Shoot dry weight (g)	Plant Height (cm)	Branches No/Plant	Shoot fresh weight (g)	Shoot dry weight (g)
T1	36.6 ± 0.88 ^h	15.0 ± 0.57 ^f	648.6 ± 5.23 ^k	114.0 ± 0.90 ^k	44.6 ± 0.88 ^g	19.0 ± 0.57 ^f	851.3 ± 4.94 ^k	166.8 ± 1.00 ^k
T2	67.6 ± 0.88 ^c	32.6 ± 0.88 ^b	1192 ± 4.33 ^d	241.4 ± 0.87 ^d	77.0 ± 0.57 ^c	44.6 ± 0.88 ^b	1886 ± 6.93 ^d	447.3 ± 1.64 ^d
T3	72.3 ± 0.88 ^b	34.3 ± 0.88 ^{ab}	1283 ± 3.60 ^c	263.0 ± 0.73 ^c	82.3 ± 0.88 ^b	47.6 ± 0.88 ^{ab}	2052 ± 6.38 ^c	491.0 ± 1.52 ^c
T4	75.6 ± 0.88 ^b	37.0 ± 1.15 ^{ab}	1457 ± 3.84 ^b	309.1 ± 0.812 ^b	86.6 ± 0.88 ^{ab}	50.0 ± 1.15 ^a	2282 ± 6.08 ^b	551.3 ± 1.46 ^b
T5	81.3 ± 0.88 ^a	39.0 ± 1.15 ^a	1583 ± 4.05 ^a	346.6 ± 0.88 ^a	91.0 ± 1.15 ^a	51.6 ± 1.20 ^a	2489 ± 6.42 ^a	621.7 ± 1.60 ^a
T6	44.6 ± 0.88 ^{fg}	18.6 ± 1.20 ^{ef}	734.6 ± 4.91 ^e	128.0 ± 0.85 ⁱ	52.6 ± 0.88 ^f	23.0 ± 0.57 ^{ef}	1055 ± 6.80 ⁱ	225.2 ± 1.45 ⁱ
T7	41.6 ± 0.88 ^g	16.0 ± 0.57 ^{ef}	688.0 ± 3.78 ^j	120.3 ± 0.66 ^j	47.6 ± 0.88 ^g	20.0 ± 0.57 ^f	890.3 ± 4.97 ^j	186.2 ± 1.06 ^j
T8	47.3 ± 0.88 ^f	20.3 ± 1.20 ^{de}	810.0 ± 4.35 ^h	142.5 ± 0.76 ^h	55.6 ± 0.88 ^f	25.3 ± 0.88 ^e	1154 ± 4.35 ^h	250.1 ± 0.94 ^h
T9	52.3 ± 0.88 ^e	24.0 ± 1.15 ^{cd}	849.3 ± 6.11 ^g	151.3 ± 1.09 ^g	61.3 ± 0.88 ^e	31.0 ± 1.15 ^d	1377 ± 7.00 ^g	301.4 ± 1.53 ^g
T10	60.3 ± 0.88 ^d	25.3 ± 0.88 ^c	952.0 ± 5.50 ^f	178.8 ± 1.03 ^f	67.6 ± 0.88 ^d	34.3 ± 0.88 ^{cd}	1509 ± 5.50 ^f	342.4 ± 1.24 ^f
T11	63.3 ± 0.88 ^{cd}	27.6 ± 0.88 ^c	1019 ± 4.91 ^e	193.0 ± 0.93 ^e	72.6 ± 0.88 ^c	37.6 ± 0.88 ^c	1624 ± 6.35 ^e	372.3 ± 1.45 ^e
ANOVA <i>p</i>	***	***	***	***	***	***	***	***

Levels of significance are represented by *** $p < 0.001$. For each parameter in the year, different letters within the column show significant differences between the treatments and control according to Tukey's HSD test at $p < 0.05$. T1, T2, T3, T4, T5, T6, T7, T8, T9, T10, and T11 are control, 5 mg L⁻¹ Fe-NPs, 10 mg L⁻¹ Fe-NPs, 5 mg L⁻¹ Fe-NPs HA, 10 mg L⁻¹ Fe-NPs HA, 100 mg L⁻¹ FeSO₄, 200 mg L⁻¹ FeSO₄, 100 mg L⁻¹ EDDHA, 200 mg L⁻¹ EDDHA, 100 mg L⁻¹ EDTA, and 200 mg L⁻¹ EDTA, respectively.

3.3. Essential Oil Yield

Data presented in Figure 2A–F indicate that Fe-sources supplementation significantly raised EO %, accompanied by increasing EO yield per plant and per fed. in both cuts relative to control plants (water spraying plants). The highest EO %, EO yield per plant, and EO yield per fed. were recorded by spraying Fe-NPs-HA at 10 mg L⁻¹ followed by 5 mg L⁻¹, meanwhile, the lowest values were recorded in control plants. In this regard, EO% of the 1st cut ranged from 0.132 to 0.293% based on air-dry weight, meanwhile it was 0.101 to 0.192% in the 2nd cut in the first season. On the other hand, it was from 0.137 to 0.295% in the 1st cut and from 0.103 to 0.209% in the second cut, respectively, in the second season.

Regarding EO yield per plant and per fed., the results showed that Fe-sources spraying had a significant impact on EO yield at both harvests in the first and second seasons. In most cases, the yield was slightly higher in the 1st cut than in the 2nd cut in both seasons. In the first season, the EO yield per plant and fed. in the first cut was 0.819–4.577 mL/plant and 13.374–74.737 L/fed. meanwhile the 2nd cut recorded 0.849–4.740 mL/plant and 13.869–77.396 L/fed. respectively (Figure 2). Additionally, in the second season, the EO yield/plant recorded 0.888–4.676 mL/plant in the first cut and 0.876–5.201 mL/plant in the second cut. Meanwhile, the EO yield/fed. was 14.510–76.354 and 14.313–84.924 in the 1st and 2nd cut, respectively. The highest EO yield per plant and per fed. In the 1st and 2nd cut throughout both seasons was obtained in plants sprayed with 10 mg L⁻¹ Fe-NPs-Ha and the lowest values were detected in untreated plants.

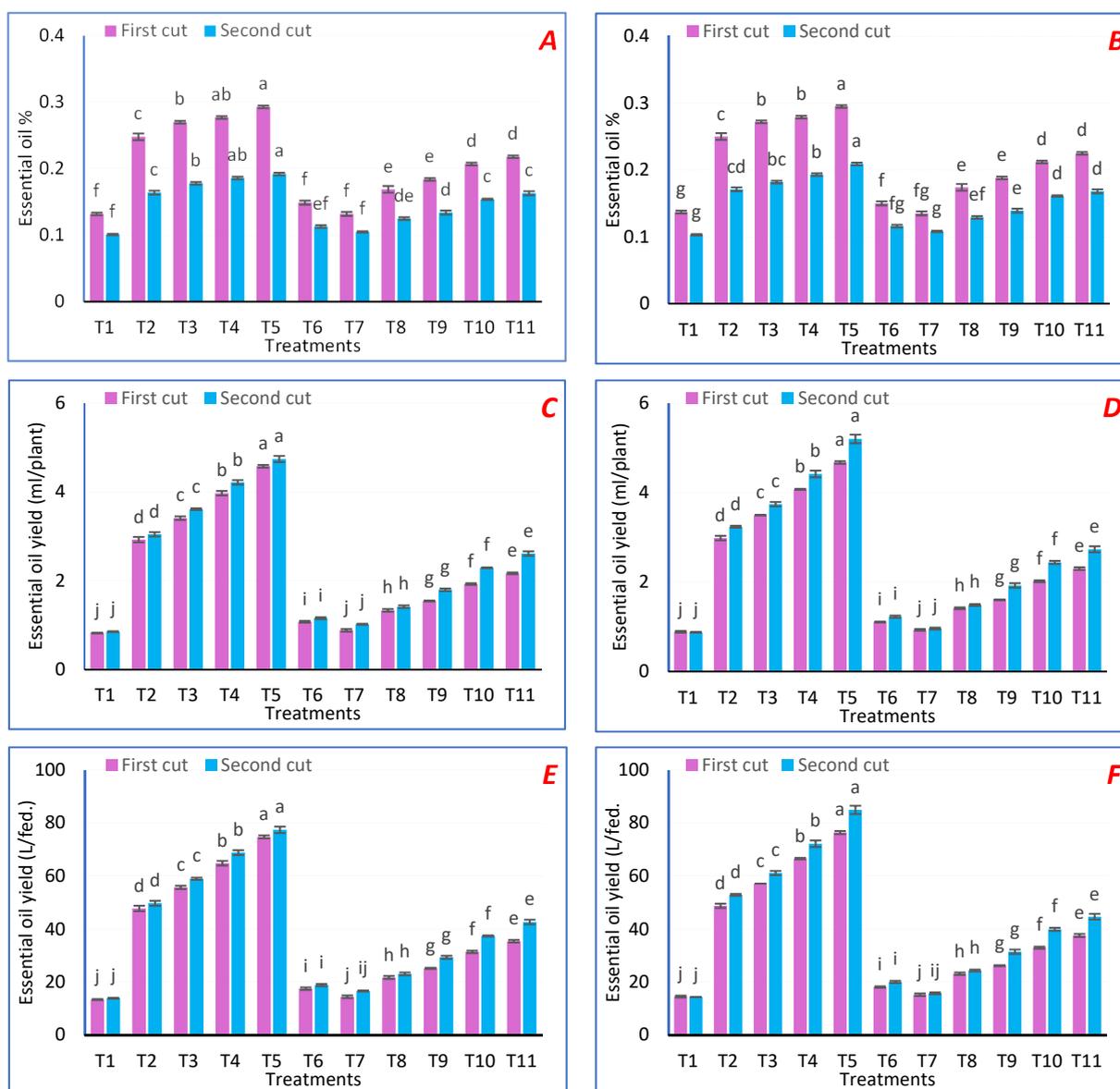


Figure 2. Effect of iron (nano, sulfate, and chelated) foliar spray on EO oil yield of Sweet Scented geranium during experimental seasons. (A) EO % in two cuts of the 1st season, (B) EO % in two cuts of the 2nd season, (C) EO yield (mL/plant) in two cuts of the 1st season, (D) EO yield (mL/plant) in two cuts of the 2nd season, (E) EO yield (L/fed.) in two cuts of the 1st season, (F) EO yield (L/fed.) in two cuts of the 2nd season. Means of three replicates are presented with \pm SE. For each parameter in the year, different letters within the column show significant differences between the treatments and control according to Tukey's HSD test at $p < 0.05$. T1, T2, T3, T4, T5, T6, T7, T8, T9, T10, and T11 are control, 5 mg L^{-1} Fe-NPs, 10 mg L^{-1} Fe-NPs, 5 mg L^{-1} Fe-NPs HA, 10 mg L^{-1} Fe-NPs HA, 100 mg L^{-1} FeSO_4 , 200 mg L^{-1} FeSO_4 , 100 mg L^{-1} EDDHA, 200 mg L^{-1} EDDHA, 100 mg L^{-1} EDTA, and 200 mg L^{-1} EDTA, respectively.

3.4. Chemical Composition of Essential Oils

Rose-scented geranium EO was slightly light green with a 0.889 g/mL density. The data belonging to qualitative and quantitative constituents of EO, collected from the 1st and 2nd cuts during the 2019 season of RSG herbs subjected to Fe-sources foliar application were identified (Tables 4 and 5). In total, 11 constituents were detected in EO accounting for 86.04% and 91.55% of the total EO in the 1st and 2nd cut respectively. A comparison of the entire set of EO analytical data showed significant variations in the EO's qualitative and quantitative composition as a result of the use of Fe-sources.

Table 4. Effect of iron (nano, sulfate, and chelated) foliar spray on essential oil active constituent's retention time (RT) and percentage (area %) of Sweet Scented geranium in the first cut during the 2019 experimental season. Means of three replicates are presented with \pm SE.

Treatments	α -Pinene		Myrcene		Isomenthone		Linalool		Citronelyl Formate		Geranyl Formate		Citronelol		Geraniol		Geranyl Butrate		Eugenol		β -Caryophyllene		Unknown Constituents	C/G Ratio
	RT	Area%	RT	Area%	RT	Area%	RT	Area%	RT	Area%	RT	Area%	RT	Area%	RT	Area%	RT	Area%	RT	Area%	RT	Area%	Area%	
T1	2.10	0.36	3.81	1.46	5.12	4.29	5.34	4.21	6.06	7.92	6.83	8.00	7.48	21.43	8.22	21.02	8.50	1.66	10.50	13.23	11.43	2.46	13.96	0.933
T2	2.03	0.35	3.72	0.82	5.01	3.99	5.25	5.98	5.93	5.91	6.70	5.33	7.33	22.42	8.07	19.09	8.35	9.23	10.31	8.26	10.95	4.20	14.42	0.817
T3	2.18	0.54	3.89	0.73	5.20	5.99	5.41	5.21	6.12	8.75	6.87	7.78	7.50	25.29	8.23	23.79	8.50	1.75	10.48	10.51	11.09	1.38	8.28	1.005
T4	1.80	0.53	3.45	1.04	4.71	6.60	4.95	8.01	5.62	9.11	6.37	7.04	6.98	25.58	7.71	23.05	7.98	1.15	9.90	6.21	10.33	1.49	10.19	1.107
T5	2.77	1.37	4.20	1.28	5.36	4.00	5.57	5.09	6.29	5.59	7.07	7.41	7.71	24.93	8.47	27.58	8.98	1.70	10.70	10.01	11.30	2.25	3.48	0.814
T6	2.24	0.87	3.99	0.36	5.33	4.72	5.55	6.38	6.25	5.58	7.03	5.48	7.63	18.29	8.42	30.21	8.67	2.57	10.69	8.61	11.32	2.75	14.18	0.621
T7	2.24	0.59	4.19	0.54	5.32	4.20	5.54	6.01	6.26	6.15	7.04	7.55	7.64	19.15	8.41	26.66	8.68	3.07	10.70	11.56	11.33	5.01	9.51	0.678
T8	2.13	0.13	3.53	0.60	4.62	5.50	4.88	5.41	5.58	8.72	6.34	7.54	7.00	22.53	7.75	26.87	7.99	1.53	9.91	8.67	10.52	2.58	9.92	0.882
T9	1.73	0.50	3.34	0.45	4.64	4.94	4.90	5.94	5.60	7.87	6.36	5.88	7.02	20.54	7.80	29.54	8.03	1.64	9.93	9.79	10.50	1.99	10.92	0.766
T10	2.06	0.30	3.83	0.12	5.16	3.36	5.41	6.03	6.15	7.09	6.93	9.14	7.59	23.89	8.37	27.73	8.87	2.35	10.61	10.82	11.55	1.90	7.27	0.787
T11	2.05	0.72	3.49	0.19	5.12	4.90	5.36	6.55	6.06	5.43	6.84	6.17	7.46	21.71	8.25	36.88	8.74	0.43	10.47	6.47	11.08	1.43	9.12	0.624

T1, T2, T3, T4, T5, T6, T7, T8, T9, T10, and T11 are control, 5 mg L⁻¹ Fe-NPs, 10 mg L⁻¹ Fe-NPs, 5 mg L⁻¹ Fe-NPs HA, 10 mg L⁻¹ Fe-NPs HA, 100 mg L⁻¹ FeSO₄, 200 mg L⁻¹ FeSO₄, 100 mg L⁻¹ EDDHA, 200 mg L⁻¹ EDDHA, 100 mg L⁻¹ EDTA, and 200 mg L⁻¹ EDTA, respectively.

Table 5. Effect of iron (nano, sulfate, and chelated) foliar spray on essential oil active constituent's retention time (RT) and percentage (area %) of Sweet Scented geranium in the second cut during the 2019 experimental season. Means of three replicates are presented with \pm SE.

Treatments	α -Pinene		Myrcene		Isomenthone		Linalool		Citronellyl Formate		Geranyl Formate		Citronelol		Geraniol		Geranyl Butrate		Eugenol		β -Caryophyllene		Unknown Constituents	C/G Ratio
	RT	Area %	RT	Area %	RT	Area %	RT	Area %	RT	Area %	RT	Area %	RT	Area %	RT	Area %	RT	Area %	RT	Area %	RT	Area %	Area %	
T1	2.06	0.62	3.80	1.09	5.19	6.20	5.40	5.11	6.15	9.35	6.87	4.34	7.63	35.55	8.30	17.28	8.79	0.85	10.52	8.36	11.08	2.85	8.45	2.029
T2	2.11	0.48	3.88	0.77	5.22	6.66	5.44	6.31	6.16	8.75	6.91	6.27	7.55	26.07	8.28	24.41	8.54	1.83	10.50	5.25	11.11	0.76	12.44	1.071
T3	2.05	0.38	3.75	0.35	4.93	4.33	5.19	12.78	5.86	6.99	6.63	2.76	7.27	28.93	8.02	22.63	8.51	2.02	10.22	5.20	10.86	1.17	12.46	1.307
T4	2.03	0.34	4.12	0.73	5.52	5.36	5.72	5.53	6.46	8.56	7.23	5.63	7.92	34.46	8.63	17.55	8.92	2.81	10.95	9.63	11.60	3.27	6.13	2.809
T5	2.27	1.31	4.07	1.22	5.48	5.59	5.67	3.47	6.44	8.53	7.20	3.19	7.93	32.19	8.62	14.06	8.91	2.15	10.97	10.24	11.61	2.92	15.13	2.098
T6	2.26	0.42	3.99	1.03	5.39	4.20	5.56	2.69	6.35	7.31	7.07	3.09	7.86	32.94	8.51	12.50	8.81	3.61	10.85	13.33	11.43	2.46	16.42	2.096
T7	2.35	0.32	4.08	0.95	5.42	4.53	5.58	3.33	6.37	8.51	7.04	3.55	7.81	36.27	8.47	14.96	8.75	2.56	10.72	11.41	11.28	3.30	10.31	2.125
T8	2.15	0.30	3.87	0.79	5.22	4.98	5.39	2.48	6.14	7.63	6.85	3.22	7.61	34.50	8.23	11.28	8.54	2.95	10.53	13.23	11.44	4.23	14.41	2.478
T9	2.16	0.68	3.88	1.31	5.21	5.71	5.40	3.09	6.12	7.69	6.86	3.18	7.53	31.44	8.21	15.28	8.51	2.13	10.50	12.21	11.09	4.48	12.8	1.896
T10	2.04	1.07	3.47	1.41	4.62	6.98	4.86	2.76	5.55	8.04	6.29	4.91	6.96	36.97	7.63	11.89	7.92	1.88	9.85	7.78	10.80	2.49	13.82	2.409
T11	2.17	0.52	3.81	0.43	5.10	4.51	5.30	3.87	6.02	7.69	6.73	4.50	7.42	26.53	8.14	22.78	8.39	2.66	10.31	10.64	11.17	2.56	13.31	1.142

T1, T2, T3, T4, T5, T6, T7, T8, T9, T10, and T11 are control, 5 mg L⁻¹ Fe-NPs, 10 mg L⁻¹ Fe-NPs, 5 mg L⁻¹ Fe-NPs HA, 10 mg L⁻¹ Fe-NPs HA, 100 mg L⁻¹ FeSO₄, 200 mg L⁻¹ FeSO₄, 100 mg L⁻¹ EDDHA, 200 mg L⁻¹ EDDHA, 100 mg L⁻¹ EDTA, and 200 mg L⁻¹ EDTA, respectively.

Citronellol and geraniol were the main ingredients of RSG-EO with treatments in the 1st cut, accounting for 18.29–25.58% and 19.09–36.88% of the total. There were also moderate amounts of eugenol (6.21–13.23%), geranyl formate (5.33–9.14%), citronelyl formate (5.43–9.11%), linalool (4.21–8.01%), and isomenthone (3.36–6.60%), as well as very variable amounts of α -pinene (0.13–1.37%), myrcene (0.12–1.46%), geranyl butyrate (0.43–9.23%), and β -caryophyllene (1.38–5.01%). According to Table 4's findings, 5 mg L⁻¹ Fe-NPs-HA was used to produce the maximum levels of citronellol, citronelyl formate, linalool, and isomenthone. Meanwhile, the application of 10 mg L⁻¹ Fe-NPs-HA, 100 mg L⁻¹ EDTA, 200 mg L⁻¹ EDTA, and 200 mg L⁻¹ FeSO₄ correspondingly resulted in the greater amount of α -pinene, geranyl formate, geraniol, and β -caryophyllene.

Citronellol (26.07–36.97%) and geraniol (11.28–24.41%) made up the majority of RSG-EO in the second cut with all treatments (Table 5). There were also moderate amounts of eugenol (5.20–13.33%), geranyl formate (2.76–6.27%), citronelyl formate (6.99–9.35%), linalool (2.48–12.78%), isomenthone (4.20–6.98%), and very variable amounts of α -pinene (0.30–1.31%), myrcene (0.35–1.41%), geranyl butyrate (0.85–3.61%), and β -caryophyllene (0.76–4.48%). The results in Table 4 demonstrate that 5 mg L⁻¹ Fe-NPs were necessary to produce the greatest amount of geranyl formate and geraniol. Meanwhile, myrcene, isomenthone, citronellol (100 mg L⁻¹ EDTA), geranyl butyrate, eugenol (100 mg L⁻¹ FeSO₄), and β -caryophyllene (200 mg L⁻¹ EDDHA) are present in larger concentrations.

Citronellol (C), geraniol (G), and their esters are the quality features in RSG-EO. Different C/G ratio was established in RSG herbs at the 1st and 2nd cut (Tables 4 and 5). In the 1st cut, the C/G ratio (from 0.621 to 1.107), additionally, the maximum C/G ratio (1.107) was recorded in T4 after that T3 (1.005) as compared with T1 (0.933). Similarly, in the 2nd cut, the C/G ratio varied from 1.071 to 2.809, with the maximum C/G ratio documented in T4 (2.809) followed by T8 (2.478) relative to T1 (2.029).

3.5. Measurement of Chlorophyll and Its Assimilation and Chlorophyll Precursor

Foliar spraying of Fe-forms significantly improved total chlorophyll and carotenoid concentrations in RSG leaves above the control plants. It is observed from the data also that Fe-NPs in special with humic acid were most effective than other Fe-forms. The greatest chlorophyll and carotenoid concentrations were obtained after 10 mg L⁻¹ Fe-NPs-HA spraying, which increased by 136 and 70% in the first cut and by 118 and 98% in the second cut respectively, over control plants (Table 6).

Table 6 shows that application of Fe-sources especially 10 mg L⁻¹ Fe-NPs-HA significantly increased Pheo, Achl a, Chl a/Chlide, and Chl b/Chlide comparative to non-treated herbs. Additionally, Table 6 designates that porphyrin intermediate assimilation (Mg-proto, proto, and Pchlide) was considerably decreased by Fe-sources supplementation.

3.6. Measurement of Ion Levels

Data existing in Table 7 display that Fe sources supplementation significantly amplified the level of ions in plant shoots in both cuts over untreated control plants. Additionally, the data also indicate that the usage of nano-forms of iron was superior to traditional sources in increasing the ion level on plant shoots. The greatest values of nitrogen (3.33 and 3.97%), phosphorous (0.222 and 0.222%), potassium (2.07 and 2.45%), iron (443 and 534 mg g⁻¹), manganese (89.7 and 43 mg g⁻¹), and zinc (87 and 89.7 mg g⁻¹) in the first cut and second cut, respectively, were recorded when plant sprayed twice with 10 mg L⁻¹ Fe-NPs-HA relative to other treatments or control plants.

Table 6. Effect of iron (nano, sulfate, and chelated) foliar spray on chlorophyll of Sweet Scented Geranium in the first and second cuts during the 2019 experimental season. Means of three replicates are presented with \pm SE.

Treatments	First Cut								
	Total Chlorophyll (mg g ⁻¹ FW)	Total Carotenoids (mg g ⁻¹ FW)	Chl A (mg g ⁻¹ FW)	Pheo A (mg g ⁻¹ FW)	Chl a/Child	Chl b/Child	Mg Proto (μ g g ⁻¹ FW)	Proto (μ g g ⁻¹ FW)	Pchilde (mg g ⁻¹ FW)
T1	0.862 \pm 0.031 ^g	0.190 \pm 0.006 ^f	0.530 \pm 0.079 ^e	0.317 \pm 0.014 ^b	0.940 \pm 0.023 ^f	0.895 \pm 0.036 ^c	0.274 \pm 0.015 ^a	0.461 \pm 0.004 ^a	0.977 \pm 0.005 ^a
T2	1.679 \pm 0.038 ^{cd}	0.313 \pm 0.010 ^{a-c}	1.148 \pm 0.027 ^{ab}	0.476 \pm 0.034 ^a	1.926 \pm 0.062 ^c	1.677 \pm 0.066 ^b	0.209 \pm 0.001 ^{bc}	0.293 \pm 0.001 ^f	0.542 \pm 0.033 ^e
T3	1.810 \pm 0.052 ^{bc}	0.317 \pm 0.008 ^{ab}	1.204 \pm 0.022 ^a	0.481 \pm 0.004 ^a	2.281 \pm 0.020 ^b	2.226 \pm 0.092 ^a	0.188 \pm 0.002 ^{cd}	0.265 \pm 0.001 ^g	0.538 \pm 0.009 ^e
T4	1.955 \pm 0.055 ^{ab}	0.319 \pm 0.008 ^{ab}	1.217 \pm 0.075 ^a	0.508 \pm 0.021 ^a	2.428 \pm 0.039 ^{ab}	2.328 \pm 0.127 ^a	0.175 \pm 0.002 ^{cd}	0.244 \pm 0.001 ^h	0.530 \pm 0.008 ^e
T5	2.038 \pm 0.008 ^a	0.323 \pm 0.001 ^a	1.248 \pm 0.091 ^a	0.538 \pm 0.016 ^a	2.577 \pm 0.014 ^a	2.355 \pm 0.068 ^a	0.160 \pm 0.001 ^e	0.226 \pm 0.001 ⁱ	0.503 \pm 0.018 ^e
T6	1.116 \pm 0.008 ^f	0.237 \pm 0.031 ^{d-f}	0.830 \pm 0.016 ^{cd}	0.436 \pm 0.035 ^{ab}	0.987 \pm 0.026 ^{ef}	0.923 \pm 0.041 ^c	0.223 \pm 0.002 ^b	0.314 \pm 0.001 ^c	0.785 \pm 0.027 ^{bc}
T7	0.957 \pm 0.005 ^g	0.214 \pm 0.005 ^{ef}	0.767 \pm 0.010 ^{de}	0.429 \pm 0.035 ^{ab}	0.975 \pm 0.035 ^f	0.878 \pm 0.082 ^c	0.253 \pm 0.002 ^a	0.354 \pm 0.001 ^b	0.815 \pm 0.035 ^b
T8	1.256 \pm 0.025 ^f	0.255 \pm 0.011 ^{c-e}	0.892 \pm 0.053 ^{b-d}	0.435 \pm 0.017 ^{ab}	1.157 \pm 0.037 ^e	1.028 \pm 0.045 ^c	0.217 \pm 0.001 ^b	0.303 \pm 0.001 ^{de}	0.686 \pm 0.028 ^{cd}
T9	1.261 \pm 0.015 ^f	0.263 \pm 0.004 ^{be}	0.936 \pm 0.010 ^{b-d}	0.453 \pm 0.032 ^a	1.698 \pm 0.037 ^d	1.544 \pm 0.046 ^b	0.216 \pm 0.002 ^b	0.305 \pm 0.001 ^d	0.580 \pm 0.036 ^{de}
T10	1.448 \pm 0.003 ^e	0.288 \pm 0.005 ^{a-d}	1.017 \pm 0.055 ^{a-d}	0.476 \pm 0.018 ^a	1.725 \pm 0.028 ^d	1.552 \pm 0.051 ^b	0.213 \pm 0.002 ^{bc}	0.299 \pm 0.001 ^{d-f}	0.562 \pm 0.011 ^e
T11	1.562 \pm 0.007 ^{de}	0.298 \pm 0.004 ^{a-c}	1.086 \pm 0.032 ^{a-d}	0.469 \pm 0.030 ^a	1.773 \pm 0.024 ^{cd}	1.618 \pm 0.054 ^b	0.211 \pm 0.002 ^{bc}	0.296 \pm 0.001 ^{ef}	0.556 \pm 0.007 ^e
ANOVA <i>p</i>	***	***	***	***	***	***	***	***	***
Treatments	Second Cut								
	Total Chlorophyll (mg g ⁻¹ FW)	Total Carotenoids (mg g ⁻¹ FW)	Chl A (mg g ⁻¹ FW)	Pheo A (mg g ⁻¹ FW)	Chl a/Child	Chl b/Child	Mg Proto (μ g g ⁻¹ FW)	Proto (μ g g ⁻¹ FW)	Pchilde (mg g ⁻¹ FW)
T1	0.883 \pm 0.011 ^h	0.144 \pm 0.002 ^g	0.430 \pm 0.000 ^f	0.333 \pm 0.011 ^f	0.706 \pm 0.019 ^g	0.645 \pm 0.026 ^h	0.298 \pm 0.002 ^a	0.415 \pm 0.001 ^a	0.913 \pm 0.019 ^a
T2	1.650 \pm 0.008 ^c	0.263 \pm 0.004 ^{ab}	1.054 \pm 0.053 ^{ab}	0.561 \pm 0.026 ^{bc}	2.213 \pm 0.065 ^c	2.032 \pm 0.085 ^{cd}	0.198 \pm 0.002 ^d	0.278 \pm 0.001 ^e	0.567 \pm 0.018 ^c
T3	1.813 \pm 0.030 ^b	0.274 \pm 0.004 ^{ab}	1.148 \pm 0.043 ^a	0.573 \pm 0.025 ^{ab}	2.404 \pm 0.057 ^{bc}	2.237 \pm 0.106 ^{bc}	0.193 \pm 0.001 ^d	0.269 \pm 0.001 ^f	0.562 \pm 0.011 ^c
T4	1.802 \pm 0.010 ^b	0.283 \pm 0.001 ^a	1.167 \pm 0.022 ^a	0.676 \pm 0.021 ^a	2.526 \pm 0.002 ^b	2.400 \pm 0.054 ^b	0.149 \pm 0.001 ^e	0.209 \pm 0.001 ^g	0.426 \pm 0.017 ^d
T5	1.933 \pm 0.010 ^a	0.286 \pm 0.001 ^a	1.210 \pm 0.068 ^a	0.676 \pm 0.018 ^a	3.671 \pm 0.069 ^a	3.399 \pm 0.126 ^a	0.100 \pm 0.001 ^f	0.140 \pm 0.001 ^h	0.424 \pm 0.004 ^d
T6	1.132 \pm 0.024 ^g	0.179 \pm 0.005 ^f	0.674 \pm 0.032 ^e	0.444 \pm 0.033 ^e	1.087 \pm 0.038 ^{ef}	1.012 \pm 0.052 ^{fg}	0.248 \pm 0.003 ^b	0.349 \pm 0.001 ^b	0.749 \pm 0.009 ^b
T7	1.096 \pm 0.013 ^g	0.163 \pm 0.012 ^{fg}	0.667 \pm 0.016 ^e	0.437 \pm 0.018 ^{ef}	0.917 \pm 0.022 ^{fg}	0.845 \pm 0.037 ^{gh}	0.250 \pm 0.002 ^b	0.348 \pm 0.001 ^b	0.887 \pm 0.004 ^a
T8	1.272 \pm 0.012 ^f	0.193 \pm 0.001 ^{ef}	0.767 \pm 0.010 ^{de}	0.455 \pm 0.009 ^{de}	1.142 \pm 0.034 ^{ef}	1.032 \pm 0.037 ^{fg}	0.248 \pm 0.002 ^b	0.346 \pm 0.001 ^b	0.624 \pm 0.015 ^c
T9	1.398 \pm 0.040 ^e	0.210 \pm 0.008 ^{de}	0.861 \pm 0.043 ^{cd}	0.458 \pm 0.024 ^{c-e}	1.302 \pm 0.021 ^e	1.214 \pm 0.039 ^{ef}	0.243 \pm 0.001 ^b	0.338 \pm 0.001 ^c	0.606 \pm 0.005 ^c

Table 6. Cont.

Treatments	Second Cut								
	Total Chlorophyll (mg g ⁻¹ FW)	Total Carotenoids (mg g ⁻¹ FW)	Chl A (mg g ⁻¹ FW)	Pheo A (mg g ⁻¹ FW)	Chl a/Child	Chl b/Child	Mg Proto (μg g ⁻¹ FW)	Proto (μg g ⁻¹ FW)	Pchilde (mg g ⁻¹ FW)
T10	1.495 ± 0.023 ^{de}	0.230 ± 0.004 ^{cd}	0.955 ± 0.010 ^{bc}	0.556 ± 0.018 ^{b-d}	1.560 ± 0.046 ^d	1.399 ± 0.060 ^e	0.219 ± 0.001 ^c	0.309 ± 0.001 ^d	0.575 ± 0.005 ^c
T11	1.539 ± 0.006 ^d	0.249 ± 0.007 ^{bc}	1.086 ± 0.032 ^{ab}	0.569 ± 0.002 ^b	2.186 ± 0.067 ^c	1.852 ± 0.071 ^d	0.202 ± 0.002 ^d	0.280 ± 0.001 ^e	0.573 ± 0.014 ^c
ANOVA <i>p</i>	***	***	***	***	***	***	***	***	***

Levels of significance are represented by *** $p < 0.001$. For each parameter in the year, different letters within the column show significant differences between the treatments and control according to Tukey's HSD test at $p < 0.05$. T1, T2, T3, T4, T5, T6, T7, T8, T9, T10, and T11 are control, 5 mg L⁻¹ Fe-NPs, 10 mg L⁻¹ Fe-NPs, 5 mg L⁻¹ Fe-NPs HA, 10 mg L⁻¹ Fe-NPs HA, 100 mg L⁻¹ FeSO₄, 200 mg L⁻¹ FeSO₄, 100 mg L⁻¹ EDDHA, 200 mg L⁻¹ EDDHA, 100 mg L⁻¹ EDTA, and 200 mg L⁻¹ EDTA, respectively.

Table 7. Effect of iron (nano, sulfate, and chelated) foliar spray on nutrients content of Sweet Scented Geranium in the first and second cut during the 2019 experimental season. Means of three replicates are presented with ± SE.

Treatments	Cut 1						Cut2					
	N%	P%	K%	Fe (mg L ⁻¹)	Mn (mg L ⁻¹)	Zn (mg L ⁻¹)	N%	P%	K%	Fe (mg L ⁻¹)	Mn (mg L ⁻¹)	Zn (mg L ⁻¹)
T1	1.98 ± 0.026 ^e	0.150 ± 0.001 ^g	1.13 ± 0.014 ^g	144 ± 0.352 ^k	21.7 ± 0.161 ^k	21.0 ± 0.282 ^k	2.35 ± 0.014 ^f	0.150 ± 0.001 ^g	1.59 ± 0.011 ^e	152 ± 1.00 ^k	17.5 ± 0.178 ⁱ	35.6 ± 0.294 ⁱ
T2	2.83 ± 0.017 ^b	0.179 ± 0.001 ^c	1.65 ± 0.017 ^c	275 ± 0.889 ^d	43.0 ± 0.280 ^d	41.7 ± 0.115 ^d	3.37 ± 0.011 ^b	0.179 ± 0.001 ^c	2.28 ± 0.015 ^b	451 ± 0.542 ^d	25.4 ± 0.121 ^d	52.3 ± 0.161 ^d
T3	2.87 ± 0.017 ^b	0.180 ± 0.001 ^c	1.72 ± 0.014 ^b	400 ± 1.39 ^c	48.5 ± 0.060 ^c	47.1 ± 0.282 ^c	3.42 ± 0.014 ^b	0.180 ± 0.001 ^c	2.43 ± 0.011 ^a	458 ± 0.069 ^c	28.6 ± 0.103 ^c	58.9 ± 0.219 ^c
T4	2.89 ± 0.026 ^b	0.205 ± 0.001 ^b	2.03 ± 0.012 ^a	411 ± 0.987 ^b	77.5 ± 0.092 ^b	75.3 ± 0.057 ^b	3.43 ± 0.014 ^b	0.205 ± 0.001 ^b	2.44 ± 0.020 ^a	524 ± 0.744 ^b	36.7 ± 0.127 ^b	75.9 ± 0.173 ^b
T5	3.33 ± 0.014 ^a	0.222 ± 0.001 ^a	2.07 ± 0.014 ^a	443 ± 1.40 ^a	89.7 ± 0.083 ^a	87.0 ± 0.271 ^a	3.97 ± 0.017 ^a	0.222 ± 0.001 ^a	2.45 ± 0.014 ^a	534 ± 0.600 ^a	43.0 ± 0.196 ^a	89.7 ± 0.132 ^a
T6	2.18 ± 0.020 ^d	0.168 ± 0.001 ^e	1.22 ± 0.014 ^f	184 ± 0.606 ⁱ	26.0 ± 0.190 ⁱ	25.3 ± 0.127 ⁱ	2.60 ± 0.011 ^{de}	0.168 ± 0.001 ^e	2.04 ± 0.014 ^c	176 ± 0.519 ⁱ	19.4 ± 0.063 ^h	39.6 ± 0.132 ^h
T7	2.14 ± 0.017 ^d	0.161 ± 0.001 ^f	1.19 ± 0.011 ^{fg}	157 ± 0.467 ^j	23.2 ± 0.176 ^j	22.5 ± 0.161 ^j	2.54 ± 0.020 ^e	0.161 ± 0.001 ^f	1.78 ± 0.018 ^d	161 ± 0.404 ^j	17.8 ± 0.109 ⁱ	36.6 ± 0.225 ⁱ
T8	2.20 ± 0.023 ^d	0.172 ± 0.001 ^{de}	1.23 ± 0.011 ^f	191 ± 0.623 ^h	28.2 ± 0.242 ^h	27.4 ± 0.167 ^h	2.62 ± 0.014 ^{de}	0.172 ± 0.001 ^{de}	2.06 ± 0.020 ^c	198 ± 0.877 ^h	20.3 ± 0.161 ^g	41.7 ± 0.305 ^g
T9	2.23 ± 0.017 ^d	0.173 ± 0.001 ^{de}	1.24 ± 0.011 ^f	222 ± 0.207 ^g	30.6 ± 0.383 ^g	29.7 ± 0.063 ^g	2.64 ± 0.020 ^d	0.173 ± 0.001 ^{de}	2.08 ± 0.068 ^c	229 ± 0.831 ^g	21.9 ± 0.167 ^f	45.2 ± 0.254 ^f
T10	2.43 ± 0.014 ^c	0.177 ± 0.001 ^{cd}	1.35 ± 0.014 ^e	253 ± 0.900 ^f	33.5 ± 0.228 ^f	32.5 ± 0.242 ^f	2.90 ± 0.017 ^c	0.177 ± 0.001 ^{cd}	2.11 ± 0.014 ^c	388 ± 0.906 ^f	24.5 ± 0.225 ^e	50.6 ± 0.155 ^e
T11	2.49 ± 0.023 ^c	0.178 ± 0.001 ^c	1.49 ± 0.012 ^d	268 ± 1.03 ^e	40.5 ± 0.167 ^e	39.3 ± 0.150 ^e	2.97 ± 0.020 ^c	0.178 ± 0.001 ^c	2.26 ± 0.014 ^b	444 ± 0.456 ^e	24.7 ± 0.103 ^{de}	50.8 ± 0.069 ^e
ANOVA <i>p</i>	***	***	***	***	***	***	***	***	***	***	***	***

Levels of significance are represented by *** $p < 0.001$. For each parameter in the year, different letters within the column show significant differences between the treatments and control according to Tukey's HSD test at $p < 0.05$. T1, T2, T3, T4, T5, T6, T7, T8, T9, T10, and T11 are control, 5 mg L⁻¹ Fe-NPs, 10 mg L⁻¹ Fe-NPs, 5 mg L⁻¹ Fe-NPs HA, 10 mg L⁻¹ Fe-NPs HA, 100 mg L⁻¹ FeSO₄, 200 mg L⁻¹ FeSO₄, 100 mg L⁻¹ EDDHA, 200 mg L⁻¹ EDDHA, 100 mg L⁻¹ EDTA, and 200 mg L⁻¹ EDTA, respectively.

3.7. Total Carbohydrate

Data in Table 8 displayed that, in general, the spraying of Fe sources increased significantly total carbohydrate concentration in the plant shoot over untreated control plants. The highest carbohydrate concentration was documented under the treatment of foliar application with 10 mg L⁻¹ Fe-NPs-HA as compared with other treatments or untreated control plants.

Table 8. Effect of iron (nano, sulfate, and chelated) foliar spray on carbohydrates and phytopharmaceuticals of Rose Scented Geranium in the first and second cut during the second season. Means of three replicates are presented with \pm SE.

Treatments	Carbohydrates (mg g ⁻¹ FW)		Phenol (mg gallic acid g ⁻¹ DW)		Flavonoids (mg quercetin g ⁻¹ DW)		Anthocyanin (mg 100 g ⁻¹ FW)	
	Cut 1	Cut 2	Cut 1	Cut 2	Cut 1	Cut 2	Cut 1	Cut 2
T1	3.041 \pm 0.439 ^b	3.295 \pm 0.124 ^e	8.084 \pm 0.157 ^g	10.91 \pm 0.199 ^d	0.989 \pm 0.007 ^d	0.998 \pm 0.008 ^g	2.156 \pm 0.028 ^c	2.167 \pm 0.012 ^f
T2	5.091 \pm 0.143 ^a	5.143 \pm 0.081 ^{a-c}	12.99 \pm 0.199 ^{a-c}	13.82 \pm 0.124 ^{ab}	2.690 \pm 0.067 ^a	2.719 \pm 0.054 ^{b-d}	3.815 \pm 0.047 ^{ab}	3.959 \pm 0.009 ^{b-d}
T3	5.424 \pm 0.097 ^a	5.532 \pm 0.016 ^{ab}	13.73 \pm 0.264 ^{ab}	13.98 \pm 0.356 ^{ab}	2.690 \pm 0.044 ^a	2.787 \pm 0.040 ^{bc}	4.114 \pm 0.053 ^a	4.339 \pm 0.049 ^{a-c}
T4	5.557 \pm 0.025 ^a	5.604 \pm 0.047 ^{ab}	13.98 \pm 0.242 ^a	14.43 \pm 0.227 ^a	2.736 \pm 0.033 ^a	2.851 \pm 0.041 ^{ab}	4.146 \pm 0.024 ^a	4.828 \pm 0.115 ^{ab}
T5	5.965 \pm 0.416 ^a	5.971 \pm 0.020 ^a	14.27 \pm 0.264 ^a	14.83 \pm 0.530 ^a	2.762 \pm 0.047 ^a	3.007 \pm 0.012 ^a	4.238 \pm 0.224 ^a	5.183 \pm 0.023 ^a
T6	4.369 \pm 0.136 ^{ab}	4.104 \pm 0.261 ^{de}	10.95 \pm 0.318 ^{ef}	12.61 \pm 0.264 ^{bc}	1.658 \pm 0.073 ^b	2.478 \pm 0.022 ^e	3.318 \pm 0.113 ^b	2.954 \pm 0.026 ^{ef}
T7	4.315 \pm 0.063 ^{ab}	3.978 \pm 0.060 ^{de}	10.37 \pm 0.446 ^f	11.89 \pm 0.448 ^{cd}	1.425 \pm 0.042 ^c	1.429 \pm 0.040 ^f	3.250 \pm 0.018 ^b	2.786 \pm 0.032 ^{ef}
T8	4.529 \pm 0.079 ^{ab}	4.184 \pm 0.052 ^{c-e}	11.31 \pm 0.246 ^{d-f}	12.70 \pm 0.338 ^{bc}	1.840 \pm 0.038 ^b	2.559 \pm 0.023 ^{de}	3.361 \pm 0.292 ^b	3.249 \pm 0.079 ^{de}
T9	4.645 \pm 0.929 ^{ab}	4.441 \pm 0.351 ^{cd}	11.60 \pm 0.369 ^{d-f}	13.33 \pm 0.136 ^{a-c}	2.550 \pm 0.007 ^a	2.584 \pm 0.011 ^{de}	3.557 \pm 0.248 ^{ab}	3.475 \pm 0.263 ^{c-e}
T10	4.749 \pm 0.073 ^{ab}	4.737 \pm 0.356 ^{b-d}	11.96 \pm 0.102 ^{c-e}	13.66 \pm 0.408 ^{ab}	2.593 \pm 0.025 ^a	2.669 \pm 0.042 ^{cd}	3.674 \pm 0.008 ^{ab}	3.462 \pm 0.044 ^{c-e}
T11	4.883 \pm 0.033 ^a	4.785 \pm 0.323 ^{b-d}	12.59 \pm 0.213 ^{b-d}	13.69 \pm 0.220 ^{ab}	2.609 \pm 0.015 ^a	2.703 \pm 0.038 ^{b-d}	3.704 \pm 0.063 ^{ab}	3.655 \pm 0.539 ^{c-e}
ANOVA <i>p</i>	***	***	***	***	***	***	***	***

Levels of significance are represented by *** $p < 0.001$. For each parameter in the year, different letters within the column show significant differences between the treatments and control according to Tukey's HSD test at $p < 0.05$. T1, T2, T3, T4, T5, T6, T7, T8, T9, T10, and T11 are control, 5 mg L⁻¹ Fe-NPs, 10 mg L⁻¹ Fe-NPs, 5 mg L⁻¹ Fe-NPs HA, 10 mg L⁻¹ Fe-NPs HA, 100 mg L⁻¹ FeSO₄, 200 mg L⁻¹ FeSO₄, 100 mg L⁻¹ EDDHA, 200 mg L⁻¹ EDDHA, 100 mg L⁻¹ EDTA, and 200 mg L⁻¹ EDTA, respectively.

3.8. Phytopharmaceuticals

As shown in Table 8, the spraying of Fe-forms significantly increased the leaf phytopharmaceutical concentrations (phenol, flavonoid, and anthocyanin) in relation to non-treated plants. The supreme of phenols (14.27 and 14.83 mg gallic acid g⁻¹ DW), flavonoids (2.762, and 3.007 mg quercetin g⁻¹ DW), and anthocyanin (4.238, 5.183 mg 100 g⁻¹ FW) concentrations in both cuts were recorded in the plant shoot treated with 10 mg L⁻¹ Fe-NPs-HA. On the other hand, the lower levels of phytopharmaceuticals were recorded in untreated control plants in either the 1st and 2nd cuts.

4. Discussion

Around the world, iron deficiency (FED) is a significant issue that may have the desired effect on plant productivity in alkaline and calcareous soil. As a result, FED may be overcome via Fe-enriching methods, which involved conventional (sulphate or chelated) and nano-compounds supplementation. According to the results of the present investigation, foliar application of Fe-sources modifies the composition of EO, phytopharmaceuticals, and RSG-plant growth. It was also noted that the use of nano-sources specifically designed for humic acid Fe-NPs-HA offered the highest values of all examined attributes and enhanced the composition and quality of EO. Kah et al. [40] conveyed that nanofertilizers application had up to 30% more effective than traditional products. The peculiar characteristics of nano-particles, i.e., their large surface area, quick mass allocation, small size, high purity, and stability may be the cause of this observation [41]. In addition to accelerating enzymatic activities, nanoparticles also have the ability to reduce the accumulation of reactive oxygen

species and oxidative damage that is improved plant development [23]. Moreover, it is attributed to their functions in modifying gene expression linked to several plant metabolic pathways [42].

Compared to untreated control plants, plant growth was dramatically boosted by the application of Fe-sources. These results were supported by previous investigations [8,18,24]. In this regard, the performance, root growth, and leaf count of sweet basil were all enhanced by the application of Fe₃O₄-NPs (1, 2, and 3 mg L⁻¹) concentration [43]. Additionally, ryegrass and pumpkin showed improved root elongation with Fe supplementation [44]. Similar findings indicating the improved influence of Fe₃O₄ NPs on a shoot and root elongation were gathered by Zahra et al. [45]. The improvement of photosynthetic processes and nucleic acid assimilation, which is reflected in an increase in photoassimilates needed for cell division and enlargement and improved plant development, may be the cause of Fe-sources' beneficial effects on plant growth [8,18,46].

It has been demonstrated that the use of Fe-sources significantly increased RSG-EO yield. Additionally, in both cuts during the first and second seasons, Nano-Fe in particular with HA (10 mg L⁻¹) was the most successful treatment (Figure 2). Previous studies have also observed an increase in EO caused by the use of Fe-sources [7,47]. On sweet marjoram, El-Khateeb et al. [8] discovered that applying Fe-NPs boosted EO% and EO production. According to Nejad et al. [25], applying Fe-sources significantly raised EO% when compared to untreated RSG plants. The generation of carbohydrates and the buildup of plant EO were positively correlated [48]. According to the results of the current study, foliar application of Fe-sources led to a greater accumulation of total carbohydrates in the herb than the control (Table 8). As a result, FeSO₄ application enhanced the content of total carbohydrates in coriander plants, according to Abou-Sreya et al. [7]. Fe-NPs foliar treatments considerably boosted the photosynthetic rate and chemical contents (carbohydrate, flavonoids, crude protein, total fatty acids, IAA), as well as oil yield, according to Abdel Wahab and Taha [49]. Additionally, El-Khateeb et al. [8] demonstrate that the total carbohydrates concentration in plant shoots of sweet marjoram treated with Fe-NPs was markedly elevated.

Essential oils, as a secondary metabolite, are highly complex mixtures of volatile compounds. Fe-sources applications affected not only EO yield but also EO constituents. In the present study, 11 constituents were identified in RSG-EO and the main components were citronellol, geraniol, and eugenol (Tables 4 and 5). A widespread study was done on the constituents of RSG-EO, which distinguished considerable variations in their constituents worldwide. In this regard, Sharopov et al. [50] in Tajikistan identified 95.1% of RSG-EO constituents, including 79 components including citronellol (37.5%), geraniol (6.0%), caryophyllene oxide (3.7%), menthone (3.1%), linalool (3.0%), β-bourbonene (2.7%), isomenthone (2.1%), and geranylformate (2.0%).

Citronellol (C), geraniol (G), and their esters are the prime components in RSG-EO as per the prerequisites of perfumery productions [1]. The C/G is the main aspect that regulates the standard of RSG-EO for fragrance manufacturing [51]. Commonly, C/G proportion of 1:1–3:1 is satisfactory; nonetheless, the best ratio is 1:1 [1,52]. Oil of C/G ratio of over 3:1 is deliberated to be of deprived quality for fragrance manufacturing nonetheless still, it can be used for the manufacture of creams, toiletries, and fragrance-based objects at a lesser price [53,54]. The variance in the C/G ratio is probably associated with environmental factors at the harvesting, which eventually influences the assimilation of citronellol and geraniol. It is described that citronellol concentrations were greater in the warm season relative to the winter season [55].

The data herein revealed that the application of Fe sources significantly raised chlorophyll above untreated plants. In line with the current results, several researchers recognized that the application of Fe-NPs [24]; Fe-sulphate [18], EDDHA [18], and EDTA [56] increased leaves chlorophyll concentration over untreated plants. The encouragement roles of Fe on chlorophyll accumulation resulted from regulating Fe, Mg, and N uptake and increase Fe availability (Table 7), as well as regulate Chl assimilation gene expression [57], stimulation chlorophyll assimilation pathways [58] and encouraging the transformation of Mg-Proto

to Pchl_{id} and consequently Chl a and b. Moreover, Fe-sources application interferes Chl degradation as indicated in the present study (Table 6), by Pheo production and avoids the change of Mg-protot_p Pchl_{id} [59], as well as hasterin ALA assimilation [60] due to declining Mg-prot_o and prot_o accumulation. As indicated previously, Fe-NPs were superior to other Fe sources in increasing chlorophyll concentration due to: (1) accelerating a dramatic upregulation of photosystem marker genes [61,62] formation of a complex with phytoferritin (leaves iron-binding protein), leading to greater involvement in chlorophyll assimilation [63]; (2) Improving thylakoid and chloroplast metabolic pathways that sequentially rise photosynthetic activities and lessening of chloroplast ROS [58,64].

The most recent results showed that spraying with Fe sources significantly increases the levels of N, P, K, Fe, Mn, and Zn in plant shoots as compared to untreated plants. The findings of El-Sonbaty [24] for Fe-NPs, Abou-Sreya et al. [7] for FeSO₄, Erdale [65] for EDTA, and Tavallali [66] for EDDHA were in agreement with these results. In this regard, Gutierrez-Ruelas et al. [18] found that in green bean, the application of Fe sources (Fe-NPs, FeSO₄, EDDHA) increased plant Fe concentration. Likewise, 0.2% Fe-EDDHA application amplified Chl a and Chl b and induced a marginal rise in the plant tissue N content [67]. Moreover, El-Sonbaty [24] found that spraying onion plants with Fe-NPs significantly increased N, P, and K content in plant organs over control plants. The role of Fe in increasing nutrient concentration and uptake may be due to increased energy availability and increased deactivated absorption of anions in root cells that increased absorption of cations as potassium [68]. Additionally, the increase in N in plant tissues by Fe sources (Fe-NPs, FeSO₄, EDDHA) application may result from the role of Fe in the enhancement of nitrate reductase activity which is increased N uptake and accumulation [18].

Currently, the supplementation of Fe-sources improved phytopharmaceutical accumulation in plant shoots, which was in accord with previous research [25,66]. Numerous phytopharmaceuticales' assembly was documented to be increased by elicitors including Fe [69,70]. The mechanism of elicitation by Fe, was, nonetheless, diverse in different herbs, and in the majority, an 'elicitor-receptor' complex was formed and a massive range of physio-biochemical responses was demonstrated [71]. The existing data have ascertained that Fe encouraged the extra accretion of phenolic in an RSG shoot. This might be because producing signal transduction systems and activating the gene for phenyl aminolyase (PAL), a secondary metabolic pathway, speed up the assimilation of phenols. The most important bioactive molecule with a reliable antioxidant has been determined to be phenolic chemicals. They have received more attention recently since they have been shown to be more effective than ascorbic acid, tocopherol, and carotenoid [72,73]. According to earlier studies [74–76], they have a variety of biological functions, including anti-inflammatory, antioxidant, antiviral, anticarcinogenic, anti-oxidant, antispasmodic, and depressive effects. Epidemiology surveys have discovered that a substantial nutritional intake of flavonoids and phenolics is coupled with lesser rates of cancer incidence [72]. The antioxidant aptitudes of phenolic compounds are mediated by numerous approaches [77]: (1) abolish ROS/reactive nitrogen species (RNS); (2) defeat ROS/RNS assembly by hindering numerous enzymes or chelating ions occupied in ROS; (3) regulate antioxidant capacity. Like total soluble phenolic, flavonoids establish a widespread secondary metabolite with polyphenolic structures and play an imperative function in shielding biological systems alongside oxidation processes [78]. In humans, flavonoids can impede aldose reductase and are occupied in diabetic difficulties i.e., neuropathy, heart disease, and retinopathy as well as attended as antioxidant compounds that lessen the hazard of cancers [79].

5. Conclusions

In the context of sustainable agriculture, prevailing and low-cost, using pioneering nanotechnology in agriculture is considered one of the encouraging attitudes for improving plant productivity. The current outcomes display a solid confirmation of the high effectiveness of nano fertilizer on plant productivity and product quality over conventional Fe-sources. The study recommended that since Fe NPs with humic acid are naturally

non-toxic, they have been utilized as Fe-enriching fertilizers to replenish Fe levels in plants, demonstrating the significance of using Fe NPs for commercial purposes.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/agronomy12092164/s1>, Table S1: Mean of monthly climatic data of the experimental site throughout the experimental seasons.

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