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Effect of soil moisture on oil quality of rose-scented geranium (*Pelargonium graveolens*)

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To enhance the yield and quality of rose-scented geranium (*Pelargonium graveolens*) essential oil, an experiment was carried out in the subtropical conditions of North India. The crop was harvested under varying soil moisture levels (9.5-26.0%). The yield and essential oil composition of rose-scented geranium varied significantly with these moisture levels. The yield was notably higher under moderate soil moisture conditions (T4; 1.40 mL per plant) as compared to excessive moisture (T2; 1.11 mL per plant). The citronellol-to-geraniol (C/G) ratio, a key quality indicator of geranium oil, varied from 0.54 to 0.88 under different treatments, with the lowest ratios observed under higher moisture levels. In conclusion, soil moisture is crucial at harvest time. Both very low and excessive soil moisture negatively impacted the essential oil yield of rose-scented geranium.

Keywords: Citronellol, Essential oil, Geraniol, *Pelargonium graveolens*, Soil moisture

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Introduction

Pelargonium graveolens L'Her. belongs to the family *Geraniaceae* and is commonly known as rose-scented geranium. It is an important perennial aromatic crop of high value in trade. The essential oil has a rose-like odour and is extensively used in perfumery and cosmetic industries[1]. The essential oil of rose-scented geranium is largely utilised in perfumery, cosmetics, and aromatherapy all over the world. It is one of the top essential oils employed in skincare[2-4]. The leaves of rose-scented geranium are used in preparing herbal tea for stress, anxiety and easing tension, as well as in curing tonsillitis[5]. The essential oil has traditionally been used in treating dysentery and haemorrhoids, as an anti-inflammatory, in menstrual flows, and even in cancer. The French healing communities currently use it as a treatment for diabetes, diarrhoea, gallbladder problems, gastric ulcers, liver problems, sterility, and urinary stones[6]. In Chinese homeopathy, it is recognised to open up the liver chakra and help remove toxins that disrupt the body's balance[6]. A recent study showed that geranium essential oil is very helpful in reducing pain due to post-herpetic neuralgia following shingles[7]. Different origins of geranium oil have resulted in the identification of over 240 compounds to date[8]. However, the major composition of the essential oil, 60-70%, is represented by three monoterpene alcohols: citronellol, geraniol, and linalool. Apart from these marker constituents, compounds like isomenthone, menthone, nerol, *cis*- and *trans*-rose oxide, α-terpineol, α-pinene, myrcene, and β-phellandrene can be found in the essential oils of all the origin[9]. The plant is mainly distributed in South Africa, parts of the tropical region of Africa, Syria, and Australia. It is an inhabitant of arid, rocky slopes of the Cape Province of South Africa and widely cultivated in Reunion, Algeria, southern France, Spain, Morocco, Madagascar, Congo, and Russia for the production of geranium oil[1].

In India, rose-scented geranium is being cultivated in the Nilgiris and Pulney hills of Tamil Nadu, the plains of Andhra Pradesh, Karnataka, Maharashtra, and northwards in the Tarai and hills of Uttarakhand[8,10-12]. There is considerable information available on agricultural and processing practices of rose-scented geranium from the growing parts of

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India. Essential oil yield and composition of the crop is subjective to the locality of growing, age of the leaves, climatic conditions, seasons, cultivars, mode of distillation, type of distillation unit used, storage practices of oil, partial shade, part of the plant distilled, time of planting and methods adopted for planting[8,10-15].

Moreover, it is well known that moisture stress in soil induces various physiological and metabolic responses; thus, it affects plant growth, plant biomass, and secondary metabolite production[16-17]. The influence of moisture in soil on the growth and developmental processes of various plant species has been investigated[16]. However, there is little known about the influence of various levels of soil moisture on secondary metabolite yield and composition during the time of crop maturity, specifically harvesting time. In general, the harvesting of medicinal and aromatic plants is done under dry and clear weather conditions. Since there is a common understanding that during harvest, water-stressed conditions are favourable for the production of secondary metabolites in plants like essential oils[17], these general recommendations, however, need to be specifically standardised. Considering these facts, it was planned to study the influence of various levels of soil moisture in terms of oil yield and composition of rose-scented geranium at the time of crop maturity. This study optimised the soil moisture level as an indicator of appropriate harvesting time for better recovery of quality rose-scented geranium oil.

Materials and Methods

Experimental site and growing of crop

A field experiment was conducted in two consecutive cropping seasons (January to May 2020 and 2021, respectively) at CSIR-Central Institute of Medicinal and Aromatic Plant, Research Centre, Pantnagar (Udham Singh Nagar), Uttarakhand, India. The experimental site is situated between the latitude 29°N and longitude 79.38°E and at a height of 243 MASL. Soil can be categorised as mollisol, with a neutral pH level. The fresh apical stem cuttings were prepared from *P. graveolens* cv. CIM-Pawan and were planted in poly-bags containing local farm soil and farm yard manure during the first week of December in both the cropping seasons. The cuttings were kept under net house conditions and light. A month-old healthy rooted cutting was transplanted in the field in the first week of January in a plot size of 16.00 m^2 in triplicate using a completely randomized block design. A total of 64 plants were transplanted in a plot. The inter-row and intra-row spacing were 50 cm each. The recommended dose of fertilizers, i.e., N:P:K at 100:60:60 kg/ha, was applied. Before transplanting, a full dose of P, K and a half-dose of N together with vermicompost, at the rate of 2.5 t/ha, was applied. The remaining N was top-dressed in two equal splits at monthly intervals. The field was irrigated immediately after transplanting, and further irrigation was provided at monthly intervals till April and at weekly intervals in May (summer). The flood irrigation method was used to irrigate the experimental field.

Collection of soil and plant samples

Both soil and plant samples were collected from the field at the time of crop maturity during the first week of May in both cropping seasons. To see the influence of soil moisture on yield and composition of the essential oil in rose-scented geranium, the soil and plant samples were first collected under dry field conditions, and then the crop field was immediately irrigated. Further, soil and plant samples were collected at different intervals after irrigation. The treatments used in the study were T_1 (just before irrigation), T_2 (24 h after irrigation), T_3 (48 h after irrigation), T_4 (72 h after irrigation), and T_5 (96 h after irrigation). T1 was taken as control while T2 to T5 were treatments. The weight of fresh soil and plant samples were taken and then oven-dried at 50°C till the complete removal of moisture. The data obtained from fresh and dried soil and plant samples were used to calculate soil and plant moisture content under different treatments. Soil moisture percentage was calculated using the formula-

$$\text{Soil Moisture (\%)} = \frac{\text{Fresh soil weight} - \text{Dry soil weight}}{\text{Dry soil weight}} X\,100$$

Extraction of the essential oil

Fresh harvested aerial parts of *P. graveolens* from various treatments (T_1-T_5) were hydro-distilled in a Clevenger apparatus for 3 hours to extract the oil. The resulting oil was calculated in the extraction burette itself, and essential oil content (%) was calculated in volume (mL) of oil for each 100 g of fresh material while essential oil yield per plant was determined on fresh biomass of random plant. 509 g was the observed average weight of a plant. The oil samples were dehydrated with anhydrous sodium sulfate (Na_2SO_4) and were stored in a cool and dark place for analysis.

Chemical analysis of essential oil

Gas Chromatography equipped with a Flame Ionisation Detector was performed to quantify the oil composition. This analysis of essential oil was done on a gas chromatograph [Nucon (India), model 5765] enabled with DB-5 (30 m × 0.32 mm; 0.25 µm film thickness) fused silica capillary column and flame ionisation detector (FID). Hydrogen was used as a carrier gas at the flow rate of 1.0 mL/min. Temperature was programmed from 60-230°C at 3°C/min. The injector and detector temperatures were 220 and 230°C, respectively. The injection volume was 0.02 µL neat with a 1:40 split ratio.

Gas Chromatography equipped with a Mass Spectrometer was also done to identify the essential oil constituents. This analysis of the oils was conducted through a Perkin-Elmer Turbo mass Quadruple Mass spectrometer fitted with PE-5 fused silica capillary column (60 m × 0.32 mm; 0.25 µm film coating). The column temperature was programmed from 100 to 280°C at 3°C/min, using helium as a carrier gas at a flow rate of 1.0 mL/min. The injector temperature was 220°C, and MS conditions were: EI mode operating at 70 eV, and ion source temperature was 250°C.

Identification of constitutional compounds

The identification of the essential oil constituents was determined on the basis of retention indices (RI, determined with reference to homologous series of *n*-alkanes, C_8-C_{24}), co-injected with known compounds, MS Library search (NIST and WILEY), and also with the MS literature data[18]. The retention times of standards /marker constituents of already identified essential oils were also used to confirm the identities. The relative amounts of the individual constituents were calculated on the basis of relative % peak areas (FID response), exclusive of the correction factor.

Statistical interpretation

The pooled numerical experimental data of two years pertaining to soil moisture, plant moisture, content and essential oil yield were analysed through SPSS 12.0, analysis of variance (ANOVA) using randomised block design[19]. In order to study whether the identified essential oil constituents are useful in reflecting the chemical constituents associations among the different compositions obtained from different levels of soil moisture (T_1-T_5), their contents (total 12 components; ≥ 1.0%) were subjected to hierarchical cluster analysis using average method[20]. This software calculates the hierarchical clusters of a multivariate dataset on the basis of dissimilarities. The resulting dendrogram depicts the grouping of different chemical compositions as per their chemical constituents.

Results and Discussion

The results pertaining to soil moisture, plant moisture, essential oil content, and essential oil yield are presented in Table 1. The soil moisture content varied significantly (9.5-26.0%) under the different treatments (T_1 to T_5), whereas the variation in the plant moisture content (79.6-82.0%) was found to be non-significant under different treatments. The essential oil yields ranged between 0.21 and 0.27% under different treatments, which is higher than other geranium cultivars[21]. Significantly higher oil content was observed in T_4 (0.27%), which was at par with T_1 (0.25%), T_3 (0.26%), and T_5 (0.25%). However, the treatment T_2 (24 hours after irrigation) recorded significantly lower essential oil content (0.21%) as compared to the rest of the treatments. The yield of essential oil varied from 1.11 to 1.40 mL/plant under different treatments and was significantly higher in T_4 (1.40 mL/plant). The essential oil losses were 20.7% under excess moisture conditions (T_2; soil moisture 26.0%) and 9.3% under extremely low soil moisture conditions (T_1; soil moisture 9.5%). However, the best level of soil moisture for better recovery of rose-scented geranium essential oil was 19.5%, and this level was achieved after 72 h of irrigation under the subtropical conditions of north India. The results

Table 1 — Effect of soil moisture level on essential oil yield of rose-scented geranium (*Pelargonium graveolens*) under foothills of north India

Treatment	Soil moisture (%)	Plant moisture (%)	Essential oil content (%)	Essential oil yield plant^{-1} (mL)
T_1	9.5	79.6	0.25	1.27
T_2	26.0	82.0	0.21	1.11
T_3	21.0	81.8	0.26	1.33
T_4	19.5	80.7	0.27	1.40
T_5	14.7	80.2	0.25	1.30
SEm ±	0.24	0.62	0.008	0.04
CD $_{(0.05)}$	0.78	NS	0.027	0.14

T_1: Just before irrigation; T_2: 24 h after irrigation; T_3: 48 h after irrigation; T_4: 72 h after irrigation; T_5: 96 h after irrigation clearly indicated that the essential oil content suddenly dropped down in the irrigated field after 24 h (T_2; soil moisture 26.0%) and improved further when the availability of soil moisture became moderate (T_3; soil moisture 21.0% and T4; soil moisture 19.5%). Moreover, over-drying of the field (moisture from 14.7 to 9.5%) resulted in the loss of essential oil recovery. The result was similar to that reported in *Ipomoea*, where the yield was reduced in excessive wet periods, which resulted in a water surplus[22]. A similar result was reported for *Ocimum* under drought stress[23].

The oils derived from different treatments (T_1 to T_5) were analysed through GC/FID and GC/MS. In total, 45 constituents representing 94.3% to 95.8% of the entire oil compositions were identified, as summarised in Table 2. Some of the major constituents of the essential oil were geraniol (27.7-35.6%),

Table 2 — Soil moisture level vs. essential oil composition of rose-scented geranium (*Pelargonium graveolens*) grown in the foothills of north India

Compound	RI	Content (%)				
		T_1	T_2	T_3	T_4	T_5
(3*Z*)-Hexenol	845	0.5±0.15	0.2±0.06	0.2±0.06	0.2±0.00	0.2±0.00
α-Pinene	931	0.4±0.10	0.4±0.06	0.4±0.06	0.3±0.00	0.3±0.12
Sabinene	968	t	-	t	t	-
β-Pinene	972	0.1±0.12	0.2±0.06	0.2±0.06	0.2±0.06	0.2±0.00
α-Phellandrene	1000	0.1±0.04	t	t	t	-
α-Terpinene	1012	0.1±0.06	0.1±0.00	0.1±0.00	0.1±0.00	0.1±0.06
p-Cymene	1018	0.3±0.15	0.2±0.00	0.2±0.06	0.3±0.06	0.3±0.06
(*Z*)-*β*-Ocimene	1032	0.2±0.11	0.1±0.06	0.1±0.06	t	0.1±0.00
(*E*)-*β*-Ocimene	1040	0.1±0.00	0.1±0.00	t	t	-
cis-Linalool oxide†	1065	0.1±0.06	0.1±0.06	0.1±0.10	0.1±0.12	0.2±0.06
trans-Linalool oxide†	1088	0.1±0.06	0.1±0.06	t	t	0.1±0.06
Linalool	1097	4.8±0.44	3.8±1.50	3.8±0.06	3.4±0.35	3.7±0.36
cis-Rose oxide	1103	0.3±0.06	0.2±0.06	0.3±0.06	0.2±0.06	0.3±0.10
trans-Rose oxide	1123	0.1±0.06	0.1±0.00	0.1±0.06	0.1±0.06	0.2±0.06
Menthone	1148	0.3±0.06	0.3±0.00	0.3±0.06	0.3±0.00	0.3±0.00
Isomenthone	1162	6.8±0.17	5.8±0.60	6.8±0.26	6.5±0.17	6.5±0.31
Terpinen-4-ol	1176	0.1±0.06	0.1±0.00	0.1±0.00	0.1±0.06	0.1±0.06
α-Terpineol	1190	0.2±0.06	0.2±0.17	0.2±0.00	0.1±0.10	0.2±0.00
Citronellol	1225	24.3±0.95	19.2±0.55	20.9±1.54	20.6±0.66	22.5±0.65
Neral	1240	0.6±0.06	0.6±0.00	0.7±0.00	0.7±0.06	0.6±0.06
Geraniol	1254	27.7±0.72	35.6±0.83	28.6±3.16	28.8±3.32	29.2±2.00
Geranial	1267	0.7±0.15	0.9±0.26	1.1±0.44	1.0±0.17	1.3±0.31
Citronellyl formate	1278	7.6±0.63	6.1±0.50	7.7±0.51	6.8±0.95	6.8±0.25
Geranyl formate	1300	4.5±0.35	5.2±0.26	5.6±0.12	5.2±0.70	4.6±0.25
Citronellyl acetate	1352	0.1±0.00	0.1±0.00	0.1±0.00	0.1±0.00	0.1±0.00
Neryl acetate	1358	0.1±0.12	0.1±0.06	t	0.1±0.17	0.1±0.22
α-Copaene	1374	0.2±0.00	0.2±0.06	0.3±0.06	0.3±0.00	0.3±0.00
Geranyl acetate	1380	0.9±0.06	0.9±0.06	1.0±0.06	1.1±0.20	1.1±0.10
β-Bourbonene	1384	0.1±0.04	0.1±0.04	0.1±0.04	0.1±0.00	0.1±0.04
(*E*)-Caryophyllene	1420	0.6±0.06	0.7±0.12	0.7±0.06	0.8±0.10	0.8±0.15
6,9-Guaidiene	1444	0.3±0.06	0.1±0.00	0.1±0.06	0.2±0.12	0.3±0.12
α-Humulene	1450	0.2±0.00	0.2±0.00	0.2±0.00	0.2±0.00	0.2±0.06
(*E*)-*β*-Farnesene	1455	0.2±0.00	0.2±0.00	0.3±0.06	0.3±0.06	0.3±0.00
Geranyl propionate	1477	1.4±0.17	1.6±0.00	1.8±0.21	2.0±0.20	1.4±0.40
Germacrene D	1480	0.1±0.04	t	0.1±0.06	0.1±0.00	0.1±0.00
α-Selinene	1496	0.7±0.10	0.7±0.00	0.8±0.06	0.9±0.10	0.9±0.15
γ-Cadinene	1517	0.2±0.10	0.2±0.16	0.2±0.12	0.4±0.06	0.3±0.06
δ-Cadinene	1523	0.6±0.06	0.5±0.00	0.7±0.06	0.8±0.10	0.7±0.06
Citronellyl butyrate	1528	t	t	0.1±0.00	0.1±0.10	0.1±0.04
Geranyl butyrate	1566	0.1±0.00	0.3±0.17	0.4±0.12	0.5±0.23	0.2±0.00
2-Phenyl ethyl tiglate	1580	0.9±0.06	1.0±0.17	1.1±0.10	1.1±0.12	0.9±0.10
10-*epi*-*γ*-Eudesmol	1623	6.5±0.21	6.7±0.31	6.5±0.32	7.1±0.67	6.9±0.00
Geranyl valerate	1653	0.1±0.00	0.2±0.06	0.2±0.06	0.2±0.06	0.2±0.06
Citronellyl tiglate	1670	0.2±0.06	0.2±0.06	0.3±0.06	0. 2±0.00	0.2±0.06

(*Contd.*)

Table 2 — Soil moisture level vs. essential oil composition of rose-scented geranium (*Pelargonium graveolens*) grown in the foothills of north India (*Contd.*)

Compound	RI	Content (%)				
		T_1	T_2	T_3	T_4	T_5
Geranyl tiglate	1700	2.1±0.15	2.2±0.35	2.5±0.46	2.5±0.36	2.3±0.25
Class composition						
Hydrocarbons (C_{10})		1.2±0.32	1.1±0.02	1.0±0.12	0.9±0.10	1.0±0.15
Hydrocarbons (C_{15})		3.1±0.45	2.9±0.32	3.4±0.36	4.0±0.26	4.1±0.36
Oxides (C_{10})		0.7±0.06	0.4±0.10	0.6±0.10	0.5±0.21	0.7±0.26
Alcohols		64.1±0.35	65.8±1.99	60.2±1.47	60.4±3.36	62.8±1.31
Ketones		7.1±0.21	6.1±0.0	7.1±0.32	6.8±0.17	6.8±0.30
Aldehydes		1.3±0.20	1.5±0.26	1.8±0.43	1.7±0.11	1.9±0.36
Esters		18.1±1.17	18.0±1.41	20.7±1.40	19.9±2.42	18.0±0.21
Total identified (%)		95.7±1.00	95.8±0.32	94.9±0.35	94.3±1.02	95.3±0.65

T_1: Just before irrigation; T_2: 24 h after irrigation; T_3: 48 h after irrigation; T_4: 72 h after irrigation; T_5: 96 h after irrigation; †Furanoid; RI: retention index determined on DB-5 gas chromatography column; t: trace (component <0.05%)

citronellol (19.2-24.3%), citronellyl formate (6.1-7.7%), 10-*epi*-γ-eudesmol (6.5-7.1%), isomenthone (5.8-6.8%), geranyl formate (4.5-5.6%), linalool (3.4-4.8%), geranyl tiglate (2.1-2.5%), geranyl propionate (1.4-2.0%), geranial (0.7-1.3%), geranyl acetate (0.9-1.1%), and 2-phenyl ethyl tiglate (0.9-1.1%). In comparison, results showed considerable variations in their quantity of chemical constituents due to varying levels of soil moisture. The results indicated that citronellol showed a sharp decline under excess moisture conditions in the soil. However, the geraniol content was parallel to the moisture level under treatments T_1 and T3. Thereafter, it showed no major deviation with the change of moisture level in the soil. Changes noticed in the citronellol and geraniol percentages under varied moisture levels are depicted in Fig. 1.

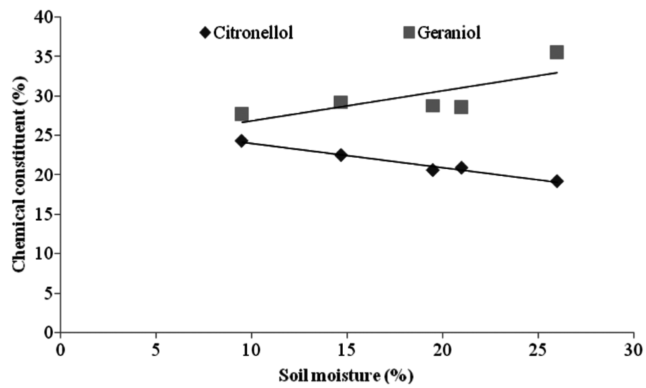

Fig. 1 — Graphical representation of the contents (%) of two major constituents of the rose-scented geranium (*Pelargonium graveolens*) essential oil as influenced by soil moisture content (%) during crop harvest under sub-tropical conditions of north India.

These were statistically validated to examine both the similarities and dissimilarities among the oil compositions obtained from different treatments. A total of 12 selected major essential oil components from Table 2 (amounting to ≥ 1%), representing 86.1 – 89.0% of the total oil compositions, were subjected to the hierarchical cluster analysis. The similarity index of the different compositions is clearly presented in the derived dendrogram (Fig. 2). The treatment T_2 (24 h after irrigation) made a separate cluster, which showed that the rose-scented geranium oil composition changed considerably under T_2. Further, the composition of rest of the treatments (T_1, T_3-T_5) made a separate cluster together. Gas chromatographic profiles of the rose-scented geranium essential oils, obtained from extremely low (T_1) and high (T_2) moisture levels, are shown in Fig. 3.

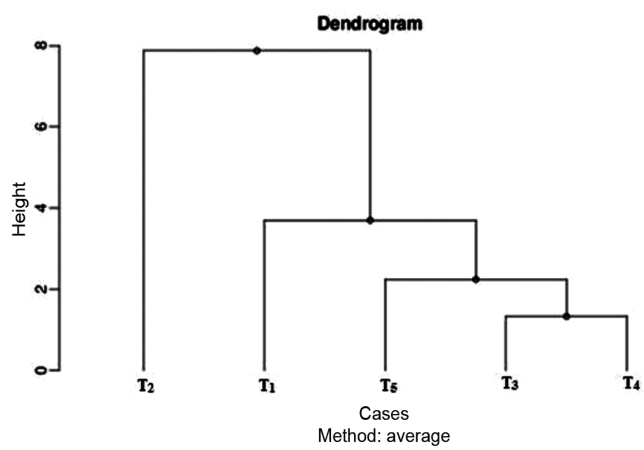

Fig. 2 — Hierarchical cluster analysis of the essential oil compositions of rose-scented geranium (*Pelargonium graveolens*) obtained under varying levels of soil moisture during crop harvest under subtropical conditions of north India. T_1: Just before irrigation; T_2: 24 h after irrigation; T_3: 48 h after irrigation; T_4: 72 h after irrigation; T_5: 96 h after irrigation.

Both citronellol and geraniol are the major compounds of geranium oil. The ratio of these two

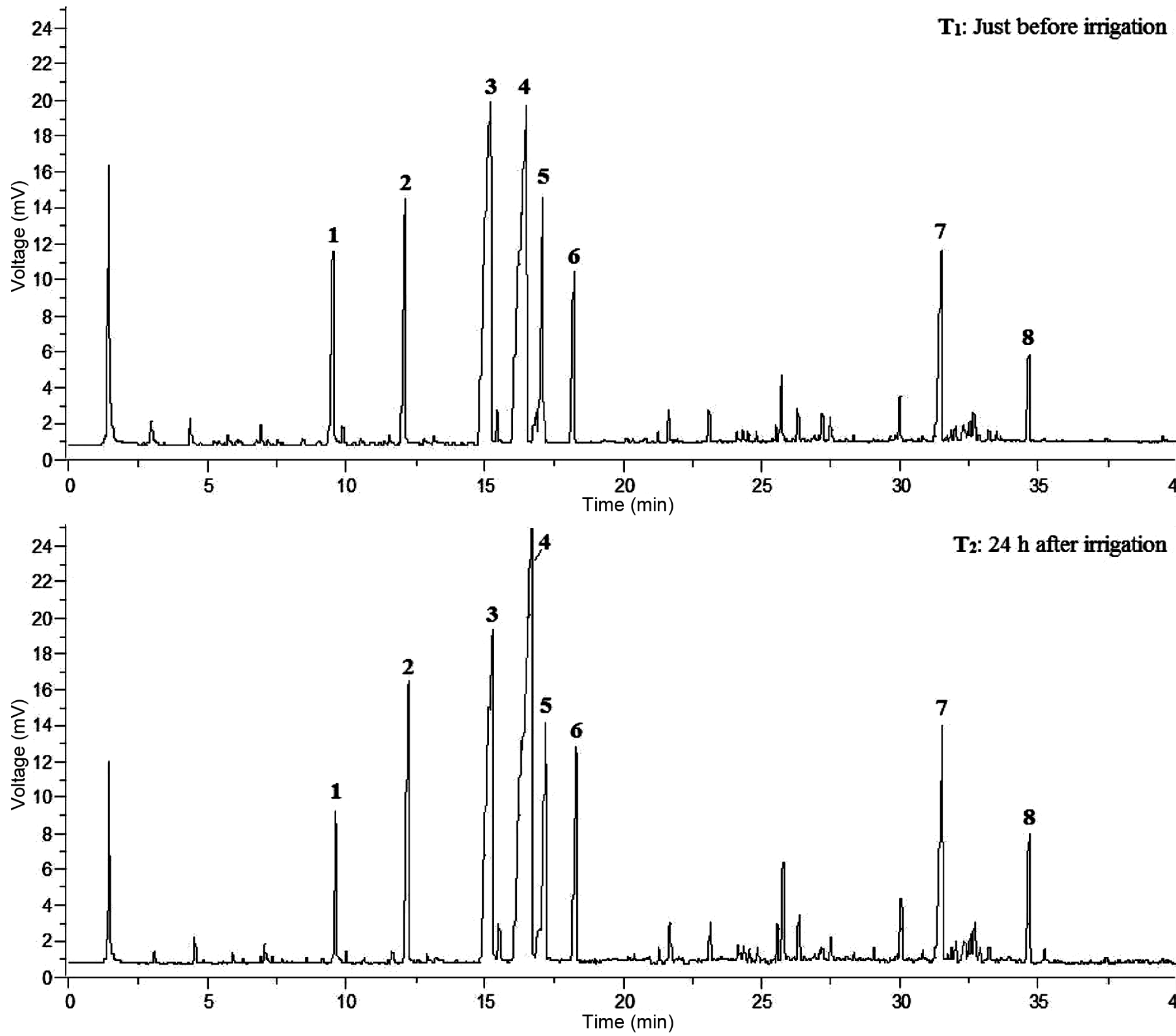

Fig. 3 — Gas chromatographic (GC/FID) profile of rose-scented geranium (*Pelargonium graveolens*) essential oils harvested under extremely low (T_1) and high (T_2) soil moisture conditions. Peaks: 1: linalool; 2: isomenthone; 3: citronellol; 4: geraniol; 5: citronellyl formate; 6: geranyl formate; 7: 10-*epi*-γ-eudesmol; 8: geranyl tiglate. For relative percentages of the constituents, see Table 2.

alcohols (C/G ratio) determines the odour value, which in turn decides the market value of the geranium oil. In the current study, the C/G ratio was found to vary from 0.54 to 0.88 under the different treatments. In general, the C/G ratio showed an opposite trend with the soil moisture content (Fig. 4). The C/G ≈ 1.0 is valued as the best quality geranium oil, whereas C/G ranged 0.5 to 2.0 is suggested for better odour quality and is acceptable in the essential oil market[24-25]. However, variations in the C/G ratio of examined rose-scented geranium oil were under the desirable range. Nevertheless, the highest C/G ratio (0.88) was observed at the lowest soil moisture level (T_1).

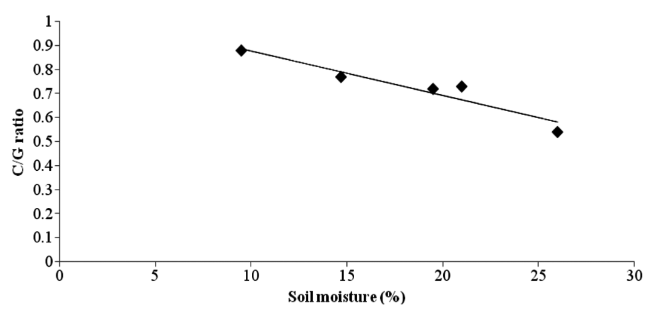

Fig. 4 — Pattern of citronellol to geraniol ratio (C/G) of rose-scented geranium (*Pelargonium graveolens*) oil under varying levels of soil moisture during crop harvest under subtropical conditions of north India.

Our results depict higher geraniol content than a recent study from Northeast India[26]. In essential oil-yielding plants, both growth and oil yield are significantly influenced by soil moisture. As soil moisture is related to water stress, studies have been done on the effect of water stress on essential oils and the physiology of plants. It was also observed that in basil, the oil yield increased by keeping plants under water stress just before harvesting[23,27]. However, in *Satureja hortensis,* severe water stress increased the essential oil content as compared to the moderate water stress condition[28]. These findings can well be correlated with the results of the present study. Both extremely low soil moisture (T_1, T_5) and excess soil moisture (T_2) were unfavourable for getting higher essential oil yield from rose-scented geranium. However, moderate soil moisture, viz. 19.5% (T_4), followed by 21.0% (T_3), and was found to be favourable for achieving a better yield of quality essential oil from rose-scented geranium. Thus, it was evident from the present and earlier studies that the overall productivity of essential oils in plants tends to boost in water-stressed conditions.

Conclusion

The present study assessed the impact of soil moisture on the oil yield and composition of rose-scented geranium. The findings are crucial for determining the ideal time to harvest the mature crop. Results emphasised the importance of optimal soil moisture levels during harvest to achieve higher essential oil yield. It was concluded that the ideal soil moisture level is around 19.5% (≈ 20.0%) for maximum essential oil yield. However, both very low (9.5%) and excessive soil moisture levels (26.0%) can significantly reduce essential oil recovery, resulting in a substantial loss of rose-scented geranium oil (9.3 and 20.7%, respectively). The quality indicator, C/G ratio, significantly decreased under high moisture conditions but improved with increasing soil moisture. Additionally, it is recommended that if it rains during crop maturity, harvesting should be delayed until the optimal soil moisture level is reached.

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Conflict of interest

The authors declare that they have no conflict of interest.

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