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Essential oil yield, composition and quality at different harvesting times in three prevalent cultivars of rose-scented geranium

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Abstract

Geranium is an economically important aromatic plant for its highly priced essential oil. The essential oil yield and composition is influenced by genetic, agronomical and environmental factors but at maturity, the right harvesting time during the day without affecting the quality is not clear. Present study was undertaken to study the change in essential oil yield and composition at different harvesting time during a day in three geranium cultivars *i.e.,* Bourbon, Bio-G-171, and CIM-Pawan. Randomised samples from three cultivars were harvested at crop maturity stage to obtain oil yield and composition of essential oil at different times (06 A.M., 10 A.M., 02 P.M., and 06 P.M.) during the day. Results indicated that essential oil yield increased significantly from 06 A.M. (0.18 %) to 06 P.M. (0.40 %) in Bourbon only and not in Bio-G-171 and CIM-Pawan. With respect to quality of essential oils, no significant quantitative variations in marker compounds was recorded. Citronellol/geraniol ratio is a quality criteria as per industry need and results unveiled high ratio in Bourbon (1.85) and CIM-Pawan (1.73) in afternoon and in Bio-G-171 (1.78) during forenoon, compared with other harvesting time during the day. The study revealed that essential oil significantly increased in cultivar Bourbon during the day but not in Bio-G-171 and CIM-Pawan cultivars. With respect to quality, considering C/G ratio, one can go for harvesting in cultivar Bourbon and CIM-Pawan in the afternoon and for cultivar Bio-G-171, harvesting in forenoon is advisable.

Key words: Rose-scented geranium, cultivars, diurnal, geranium oil, composition

Introduction

Rose scented geranium (*Pelargonium graveolens* L.) of genus *Pelargonium* L. Herit, belonging to family Geraniaceae, is one of the economically important aromatic plant from which highly prized geranium essential oil for the trade is extracted for flavour and fragrance industry. Geranium oil is highly valuable for its profound and strong rose like odour. Leaves of rose scented geranium are densely pubescent and are highly aromatic in nature. The colour of geranium oil is slightly yellowish with pleasant odour, insoluble in water and soluble in alcohol (Vijay *et al.*, 2014). Essential oil of geranium contain two types of constituents *i.e.* alcohol and esters. The marker components are citronellol, and geraniol. Geranium essential oil is parallel to rose oil owing to some identical constituents (Anon, 2006). Geranium essential oil is basically used in industries in particular in perfumery, beauty and aromatherapy industries all around the world. It is one of the quality skin care oil as it is used for opening skin pores and cleansing complexions (Miller, 2002; Peterson *et al.*, 2006). Other additional common uses of geranium essential oil are in infectious disease treatment, haemorrhoids, inflammation, serious menorrhea and even cancer. In French medical profession, it is used in the treatment of polygenic disease, diarrhoea, vesica issues, internal organ ulcers, jaundice, liver issues, sterility and urinary stones. Leaves are also utilized as herb drink to fight anxiety, relief strain, to boost blood flow, and to treat inflammation (Peterson *et al.*, 2006).

Plant growth and economic yield of an aromatic crop depends on genetic and environmental factors. Similarly, the biogenesis of secondary metabolites is controlled genetically in addition to environmental influences of specific region, agronomical conditions, harvest time, and post-harvest processes (Yi and Wetzstein, 2011). Harvesting time is very important factor to get maximum yield of quality essential oils (Carvalho-Filho *et al.*, 2006; Murray *et al.*, 1988). Therefore, optimizing harvesting time is of basic importance for higher production and quality oil. Moreover, proportion of citronellol over geraniol (C/G) is prime crucial component in geranium oil which vary when the share of any of these two changes (Verma *et al.*, 2010).Till date, it is not evident that when and in what stage the harvesting should be done in geranium for maximum essential oil yield with better quality. Therefore the present experiment was designed to optimise the right harvesting time for higher yield of quality essential oils of three prevalent geranium cultivars (Bourbon, Bio-G-171 and CIM-Pawan) grown in foothills conditions of Uttarakhand, India.

Materials and methods

Growing conditions and cultivars: Research was carried out at research centre farm of CSIR-Central Institute of Medicinal and Aromatic Plants, Pantnagar. Site was situated at the latitude 29° N and longitude 79.38° E, and at an elevation of 243 m over mean sea level. Soil is mollisol type with a pH of 7.8. Three cultivars *i.e.* CIM-Pawan, Bio-G-171, and Bourbon were used for experimental purpose. For experimental purpose, 90 days mature crop of three targeted cultivars was harvested at different times during the day.

Oil extraction: The herb part of mature geranium crop from three cultivars was harvested at different time (6:00 A.M., 10:00 A.M., 2:00 P.M., and 6:00 P.M.) during the daytime. Oil was extracted by hydro-distillation through clevenger equipment for 3 hours. The oil quantity was calculated directly within the extraction measuring device. Oil percentage was calculated as volume (mL) of oil per 100 g of plant sample. Isolated oil was dried over anhydrous Na_2SO_4 in sealed bottle in cold condition.

Essential oil analysis: The essential oil was analysed using GC-FID and GC-MS techniques. Analysis of essential oil by GC-FID technology on Thermo Fisher Trace GC-1300 mixed with TG-5 fused silica capillary column (30 m × 0.25 mm × 0.25 µm) and flame ionization detector (FID). Nitrogen at flow rate of 1.0 mL min^{-1} was used as carrier gas. The oven temperature initially risen from 60 to 23 °C at 3 °C min^{-1}. An injector and detector temperature maintained at 250 and 280 °C, respectively. The injection volume was 0.02 µL neat with a split ratio of 1:40. GC-MS performed on Clarus 680 GC coupled with Clarus SQ 8C mass spectrometer of PerkinElmer equipped with Elite-5 fused silica capillary column (30 m × 0.25 mm × 0.25 µm). The oven column temperature program was from 60 to 240 °C at 3 °C min^{-1} with initial and final hold time of 2 min. Helium was employed as carrier gas at 1.0 mL min^{-1}. And the sample was split using a ratio of 1:30. The injector and detector temperatures were held at 250 °C. Different compounds were ionised using an ionisation potential of 70 eV. Mostly compounds were identified by comparing their mass spectra (MS) data with literature data (Adams, 2007).

Statistical analysis: The statistical analysis was done by one-way ANOVA followed by Tukey's multiple comparison test using Graphpad Prism statistical software at 0.05 % probability.

Results

Essential oil yield (%): Data for yield of essential oil in three prevalent cultivars as per experimental design is presented in Table 1. The oil yield ranged from 0.28-0.35 % in cultivars CIM-Pawan and Bio-G-171 and showed no significant variation as per the timing of the harvest. However, in Bourbon cultivar, oil yield varied from 0.18-0.40 %, and showed significant variation at different harvesting timings throughout the day. Maximum yield of essential oil in cultivar Bourbon was found in evening harvested sample (0.40 %) followed by 2 P.M. (0.35 %), 10 A.M. (0.30 %) with minimum at 6 A.M. (0.18 %). Results showed, as the day proceeded, the essential oil yield (%) in Bourbon cultivar increased by 55 % compared with morning time.

Essential oil composition at different harvesting time: Composition of essential oil of three prevalent cultivars of rose-scented geranium (Bourbon, Bio-G-171 and CIM-Pawan) as per harvesting time throughout the day was probed by GC and GC-MS approach. Overall, 24 compounds detected comprising 91.50-93.92 % of total oil make-up. The qualitative & quantitative compositions along with class composition of the essential oils in three geranium cultivars at different harvesting times are presented in Table 2. Isomenthone, citronellol, geraniol, citronellyl formate and 10-epi-γ-eudesmol, were the key marker compounds identified in Geranium essential oil of all three cultivars. Results indicated varied concentration of major compounds *viz.,* geraniol (30.13-36.21 %); (31.94-34.93 %) ; (29.96-34.75 %), citronellol (19.57-22.84 %); (19.57-22.30 %); (19.64-22.38 %), citronellyl formate (7.34-8.57 %); (7.12-7.86 %); (7.39-7.72 %), 10-epi-γ-eudesmol (6.97-8.25 %); (7.68-8.15 %); (7.64-7.83 %) and isomenthone (4.77-6.47 %); (5.41-6.12 %); (5.35-6.42 %) in Bourbon, Bio-G-171, and CIM-Pawan, respectively. Based on the analysis of chromatogram, no large variation in availability of marker compounds in geranium essential oil in all three cultivars was found. As shown in Table 2, geraniol increased at slower rate upto 2 P.M. and then decreased in evening time in Bourbon but in Bio-G-171, 10 P.M. onwards, no large variation was observed and in CIM-Pawan, geraniol yield was more in morning (34.75 %) followed by afternoon time (34.00 %). In case of citronellol, the yield was more in the morning time (22.84 %) in Bourbon, afternoon (22.30 %) in Bio-G-171 and evening (22.38 %) in CIM-Pawan. Citronellyl formate was more in the morning time in Bourbon (8.57 %) and CIM-Pawan (7.72 %) and then decreased during the day time (Table 2). Concentration of 10-epi-γ-eudesmol was found more in evening hours (8.25 %) (8.15 %) (7.83 %) in all three geranium cultivars compared with morning (6.97, 7.87, 7.64 %), and afternoon (7.61, 7.74, 7.80 %). Isomenthone was more in morning in Bourbon, before noon in Bio-G-171 and in the evening in CIM-Pawan cultivar. Linalool was found more in afternoon (2.57 %) and evening time (2.38 %) in Bourbon and CIM-Pawan, respectively and before noon (2.86 %) in Bio-G-171 (Table 2).

Table 1. Diurnal variation in essential oil yield in three Geranium cultivars

Harvesting time	Essential Oil yield (%)		
	Bourbon	Bio-G-171	CIM-Pawan
6:00 A.M.	0.18c	0.30	0.28
10:00 A.M.	0.30b	0.35	0.35
2:00 P.M.	0.35ab	0.28	0.35
6:00 P.M.	0.40a	0.35	0.35
C.D. (at 5 %)	0.06	NS	NS

NS-non-significant; *means followed by the same letter are not significantly different at P<0.05

C/G ratio at different harvesting times: Citronellol, geraniol and their esters are the quality determinants in geranium essential oil. Different C/G ratio was found in geranium oil of Bourbon, Bio-G-171, and CIM-Pawan cultivars at different harvesting time (Table 3). In cultivar Bourbon, C/G ratio was higher in afternoon (1.85) followed by evening (1.77) compared with morning (1.32) and forenoon (1.52). On the other hand, in cultivar Bio-G-171, the citronellol and geraniol ratio ranged from 1.53 to 1.78, the highest C/G ratio was found in forenoon (1.78) followed by evening (1.69) as compared with morning (1.58) and afternoon (1.53). Similarly, in cultivar CIM-Pawan, C/G ratio varied from 1.37 to 1.73, with highest C/G ratio recorded in afternoon (1.73) followed by morning (1.60) compared with forenoon (1.37) and evening (1.51) during the day.

Table 2. Essential oil composition under different harvesting times in three geranium cultivars

S.No.	Compounds	Bourbon				Bio-G-171				CIM-Pawan			
		6:00 A.M.	10:00 A.M.	2:00 P.M.	6:00 P.M.	6:00 A.M.	10:00 A.M.	2:00 P.M.	6:00 P.M.	6:00 A.M.	10:00 A.M.	2:00 P.M.	6:00 P.M.
1	α-Pinene	0.17	0.12	0.17	0.18	0.17	0.10	0.06	nd	0.33	0.30	0.17	0.27
2	Limonene	0.10	0.09	0.18	0.13	nd	0.11	0.07	0.06	0.16	0.16	0.13	0.14
3	Linalool	2.34	1.97	2.57	2.31	2.55	2.86	2.80	2.17	2.11	2.30	1.69	2.38
4	*cis*-Rose-oxide	0.23	0.19	0.20	0.19	0.23	0.18	0.21	0.20	0.20	0.29	0.19	0.26
5	*trans*-Rose-oxide	0.14	0.08	0.27	nd	0.11	0.08	0.10	0.09	0.09	0.16	0.09	0.15
6	Isomenthone	6.47	5.22	4.77	5.32	5.99	6.12	5.42	5.41	5.56	5.77	5.35	6.42
7	α-Terpineol	nd	0.09	0.12	0.14	0.10	0.14	0.13	0.10	0.09	0.10	0.09	0.11
8	Citronellol	22.84	22.54	19.57	19.80	20.23	19.57	22.30	20.62	21.67	21.92	19.64	22.38
9	Neral	0.89	0.75	0.79	0.78	0.82	0.78	0.81	0.76	0.69	0.75	0.81	0.82
10	Geraniol	30.13	34.16	36.21	34.95	31.94	34.76	34.13	34.93	34.75	29.96	34.00	33.79
11	Citronellyl formate	8.57	7.34	7.39	7.77	7.79	7.13	7.86	7.12	7.72	7.65	7.43	7.39
12	Geranyl formate	4.80	4.12	4.04	3.94	4.63	3.95	3.80	4.33	4.37	4.06	4.71	3.88
13	Citronellyl acetate	0.18	0.18	0.17	0.18	0.05	ND	0.05	0.05	0.17	0.19	0.20	0.16
14	Geranyl acetate	0.77	0.64	0.58	0.63	0.78	0.43	0.64	0.66	0.60	0.76	0.73	0.56
15	β-Caryophyllene	0.56	0.52	0.55	0.61	0.76	0.51	0.45	0.51	0.58	0.76	0.67	0.42
16	Citronellyl propionate	nd	nd	nd	0.09	0.15	0.18	0.14	0.10	0.07	0.17	0.19	0.10
17	Germacrene-D	0.58	0.80	0.84	0.93	0.74	0.93	0.34	0.71	0.65	1.07	1.01	0.66
18	*cis*-β-Guaiene	0.69	0.70	0.74	0.78	0.87	0.69	0.41	0.80	0.74	1.05	0.94	0.52
19	Geranyl butyrate	0.69	0.31	0.50	0.42	0.28	0.26	0.29	0.26	0.27	0.53	0.66	0.49
20	2-Phenyl-ethyl tiglate	1.12	1.22	1.14	1.24	1.33	1.17	1.25	0.27	1.24	1.06	1.21	1.14
21	10-*epi*-γ-Eudesmol	6.97	7.84	7.61	8.25	7.87	7.68	7.74	8.15	7.64	7.77	7.80	7.83
22	Geranyl valerate	1.75	1.66	1.71	1.72	1.81	1.71	0.65	1.72	1.53	1.79	1.67	1.60
23	Citronellyl tiglate	0.33	0.31	0.32	0.34	0.31	0.35	0.30	0.30	0.28	0.36	0.35	0.28
24	Geranyl tiglate	2.53	2.69	2.51	2.64	2.68	2.47	2.88	2.56	2.41	2.57	2.58	2.07
Class composition													
	Monoterpene hydrocarbons	0.27	0.21	0.35	0.31	0.17	0.21	0.13	0.06	0.49	0.46	0.3	0.41
	Oxygenated monoterpenes	83.78	83.47	82.86	82.46	81.78	82.14	83.76	81.65	83.82	80.39	81.59	83.98
	Sesquiterpine hydrocarbons	1.83	2.02	2.13	2.32	2.37	2.13	1.2	2.02	1.97	2.88	2.62	1.6
	Oxygenated sesquiterpenes	6.97	7.84	7.61	8.25	7.87	7.68	7.74	8.15	7.64	7.77	7.8	7.83
	Total identified (%)	92.85	93.54	92.95	93.34	92.19	92.16	92.83	91.88	93.92	91.50	92.31	93.82

nd-not detected

Table 3. C/G ratio in essential oil influenced by harvesting time in three geranium cultivars

Bourbon				Bio-G-171				CIM-Pawan			
6:00 A.M.	10:00 A.M.	2:00 P.M.	6:00 P.M.	6:00 A.M.	10:00 A.M.	2:00 P.M.	6:00 P.M.	6:00 A.M.	10:00 A.M.	2:00 P.M.	6:00 P.M.
1.32	1.52	1.85	1.77	1.58	1.78	1.53	1.69	1.60	1.37	1.73	1.51

Discussion

Essential oil yield in three prevalent geranium cultivars was influenced by harvesting time. Oil yield variation due to harvest time have been observed and reported by many researchers in several other aromatic crops (Schwob *et al.*, 2004; Masada *et al.*, 2007; Argyropoulou *et al.*, 2007; Ebrahimi *et al.*, 2008; Callan *et al.*, 2007). Ceylan (1995) reported that essential oil yield vary during the daytime. Essential oil yield normally increased from morning to afternoon and then decreased towards evening in the essential oil bearing plants. In our study, it varied only in Bourbon cultivar from 0.10 percent to 0.40 %. Plants are under the influence of many environmental conditions throughout the day, therefore harvest timing might cause variations in essential oil. This point suggests the existence of diurnal variability (Arabaci *et al.*, 2015). In *Cistus monspeliensis* L., essential oil yield was more at 6 P.M. and essential oil yield varied during the day (Angelopoulon *et al.*, 2002). Malatova *et al.* (2011) reported that change in air temperature caused diurnal changes in essential oil yield recovery. Kaya *et al.* (2015) confirmed that throughout the day, essential oil yield remarkably varied. According to Melo *et al.* (2011) essential oil yield is a function of temperature, light intensity and relative humidity throughout the day. Differences in essential oil yield due to different harvesting time were earlier also reported in several plants (Oliveira *et al.*, 2012; Melo *et al.*, 2011; Silva *et al.*, 2016).

For quality oil purpose, essential oil composition showed slight quantitative variation in marker compounds among three varieties. This may be due to difference in photosynthetic ability (Baby *et al.*, 2009), category and effect of environmental stress (Sampaio *et al.*, 2016) as well as area and bulk of glandular trichomes (Afshari and Rahimmalek, 2018) at different phenological stages which alter the biosynthesis and buildup of volatile oil. Variability of marker compounds in essential oil seems to be due to expression and activity of concerned genes and enzymes in plant secondary metabolism which vary at different biological process stages. Considering these facts, variation in oil yield and composition throughout plant season might dissent in keeping with season and place (Boukhris *et al.*, 2015). In present study, 24 constituents were identified in geranium oil of three cultivars and the marker constituents were isomenthone, citronellol, geraniol, citronellyl formate and 10-epi-γ-eudesmol (Table 2). Harvesting time during the daytime may be used to adjust the chemical profiling in geranium essential oil in different cultivars. After comparing the geranium oil composition in three cultivars at four different harvest times of the day, we observed that relative composition of marker compounds showed small changes. The data is in support with the work of Eiasu *et al.* (2008) and Malatova *et al.* (2011).

Citronellol, geraniol and their esters are the major ingredients in geranium oil as per requirement of perfumery industries (Kulkarni *et al.*, 1997). The C/G quantitative relation is the major factor that determines the standard of geranium essential oil for fragrance industry (Saxena *et al.*, 2008). C/G proportion of 1:1-3:1 is satisfactory but the best proportion is 1:1 (Verma *et al.*, 2010; Mosta *et al.*, 2006; Southwell and Stiff, 1995). Oil of C/G proportion of more than 3:1 is considered to be of destitute quality for the aroma industry but still it can be utilized by other industries for the production of creams, cleansers, toiletries and fragrance based treatment items at lower cost (Peterson *et al.*, 2006; Weiss, 1997). The difference in C/G ratio may be due environmental factors such as light and temperature at the time of harvesting which ultimately influence the concentration of citronellol and geraniol and rate of synthesis in geranium oil. It is reported that citronellol concentration was more in warm season as compared to winter season (Rao *et al.*, 1996). Other reports on rose scented geranium indicate that citronellol concentration increased in all through the late winter-spring (Doimo *et al.*, 1999).

From present study it could be concluded that harvesting time during the day had no significant effect on essential oil yield in Bio-G-171 and CIM-Pawan cultivars of geranium, however had significant effect in Bourbon cultivar. For Bourbon cultivar, harvesting should be done in afternoon for maximum essential oil yield. Qualitatively, there was no significant variation in marker constituents of essential oils in geranium cultivars but for industry need, considering C/G ratio, harvesting of Bourbon and CIM-Pawan can be done in afternoon while the cultivar Bio-G-171 can be harvested in forenoon.

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