ISSN: 2637-7721

MEDDOCS Open Access Publisher

Journal of Plant Biology and Crop Research

Open Access | Research Article

Agro-Morphological Characters, Essential Oil Content and Essential Oil Yield of Rose-Scented Geranium (*Pelargonium Graveolense L.*) Under different environmental Conditions of Ethiopia

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Received: Jul 19, 2023

Accepted: Aug 16, 2023

Published Online: Aug 23, 2023

Journal: Journal of Plant Biology and Crop Research

Publisher: MedDocs Publishers LLC

Online edition: http://meddocsonline.org/

Keywords: Rose-scented geranium; Aromatic and medicinal herbs; Essential oil; Ethiopia; Leaf yield; Agro-ecology.

Abstract

Rose-scented geranium (*Pelargonium graveolens* L.) is an erect, multi-branching, perennial, aromatic and medicinal herb that belongs to the family Geraniaceae. Its foliage produces top high value essential oils that are highly demanded in fragrance, perfumery and cosmetic industries as a substitute of the rose scent. To get optimum economic reverse from this top-tier cash crop, it is imperative to assess and identify the best growing environment (growing condition for its cultivation). Therefore, this study was conducted in six locations in order to determine the most suitable growing conditions for rose-scented geranium. Data on plant height, branches number/plant, internodes number/plant, foliage to stem ratio, leaves number/plant, fresh foliage yield/plant and fresh foliage yield/ha, essential oil (EO) content and EO yield were examined. The results show that growing location had a highly significant effect ($P < 0.01$) on all agro-morphological characters, EO content and EO yield of rose-scented geranium. In addition, among selected environmental variables, association of altitude and temperature with plant height and number of internodes/plant respectively were found to be very highly significant. The respective fresh foliage yield/plant, foliage yield/ha, EO content, and EO yield/ha ranged from 0.3 to 0.6 kg, 18.5 to 29.5 t, 0.05 to 0.11%, and 10 to 22 kg depending on growing locations. Generally growing conditions with clay soil characteristic having a pH of 6, and average annual temperatures between 10 and 22°C are more suitable for field cultivation of rose-scented geranium to efficiently extract its EO.

Introduction

Rose-scented geranium (*Pelargonium graveolens* L. Hérit) is an erect, multi-branching, perennial, commercially important aromatic and medicinal herb of the Geraniaceae family native to south Africa [1]. There are over 750 varieties of cultivated geraniums all around the world [2], most of which are grown for ornamental purposes [3,4]. P. graveolens is among essential oil producing species of the genus [5] and mainly cultivated in Egypt, China, Comoros, India, France, Morocco, Algeria, Tunisia and South Africa for the extraction of EO [6].

Rose-scented geranium is being cultivated for the production of EO mainly from its leaves and in small quantities from flowers and stalks through stem or hydro distillation, which imparts fine rose, citrus and mint like odors [7-9]. The main constituents of the essential oil are citronellol, geraniol, geranyl acetate, limonene, phenyl ethyl alchol, linalool, iso-menthone, citronellyl formate, geraniolformate, epi-α-cadinol, 10-epi-γ-eudesmol, and guaia-6,9-diene [1,2,9].

Essential oil of rose-scented geranium is one of the most expensive and its importance has been raised over the past few years [10]. It has wide-ranging applications in the perfumery, aromatherapy, fragrance, cosmetic, pharmaceutical, and food industries [1]. It is one of the best skincare oils because it is good in opening skin pores and cleaning oily complexions [11,12]. It is also used for the treatment of heavy menstrual flows and menopause problems [13,14], dysentery, hemorrhoids, inflammation, cancer, diabetes, diarrhoea, gallbladder problems, gastric ulcers, jaundice and liver problems [15,3]. The EO is also useful for controlling of mite-transmitted dermatoses and infectious diseases, eczema and athlete foot problems [16]. Leaves are used in a form of herbal tea to de-stress, to fight anxiety, to ease tension, to improve circulation and to cure tonsillitis [12]. Besides, the whole plant is found to be effective for phytoremediation and sanitary cases [17,18]. The Nematicidal and pesticidal activity of its EO is also reported [19,20].

Regarding the effect of environmental and abiotic variables, there are several studies show that the growth, yield, and quality of rose-scented geraniums are influenced by a variety of factors. Growing location and seasonal changes of the area, time and strategies of harvesting, dry period and drought stress, latitude, longitude, altitude, soil pH, and soil type are the most variables that affecte quality and yields of rose-scented geraniums [21-24].

Transparency Market Research (2020) [25] reported that the steady rise of rose oil production from 135 metric tons in 2010 to 184 metric tons in 2016 is attributable to the rising awareness about the health benefits associated with the product. The rise in global demand for its EO as well as the existence of conducive investment climates in Ethiopia has attracted the interests of investors for the large scale cultivation of rose-scented geranium in Ethiopia. However, the research on rose-scented geranium is neither complete nor conclusive under Ethiopian condition, which is rich in diverse agro climatic conditions [26]. The availability of limited information is one of the major hindrances to exploit the entire potential of the plant. Accordingly, implementation of well-planned research and experimentation as well as displaying the optimum production conditions are pre-requisite steps for effective production and sustainable build-up of the rose-scented industry. Therefore, this investigation was designed with the objective to assess the influence of different growing environments with variety of ecological parameters on agro-morphological characters, EO content and EO yield of rose-scented geranium for its proper cultivation and utilization.

Material and Methods

Description of the study area

The assessment was conducted in six locations of Ethiopia (Figure 1). The ecological descriptions of the testing locations are summarized in table 1. The testing locations are typically different in altitude, soil type, soil pH, rainfall and temperature.

Cultural practices

Soft stem tip cuttings with 15 cm length were taken from 6 month old disease free mother plants of Wondo Genet Agricultural Research Center botanical garden for seedling preparation. Seedlings were raised in the nursery for two months in polyethylene bags before being transplanted to the field experimental plots. Plot size having area of 12.96.8 m^2 with 3.6 m length and 3.6 m width was used for national adaptation testing with four replications for two years. During experimentation, all field horticultural practices were performed as required. Harvesting was made four and a half months after transplanting.

Data collection and essential oil content determination

Data on plant height, number of branches/plant, number of leaves/plant, fresh leaf yield/plant, leaf to stem ratio, internodes number/plant, fresh leaf yield/ha, EO content and EO yield were collected and analysed. EO content was determined on a fresh weight basis from 300 g of composite leaves harvested from the three middle rows of a plot. EO was determined by hydro-distillation according to the procedures of Guenther (1972). Hydro distillation is a distillation method in which the plant material gets distilled in direct contact with the boiling water. Heat was provided by electro mantle. The emerging vapour from the flask containing the volatile essential oil was led to a condenser for condensation and collected in the oil separate unit.The essential oils were collected, dehydrated, and determined in % on dry weight basis (w/w) in the following manner.

$$Essential\ oil\ content\ (\%\ w/w)\ = \frac{Woil\ X\ 100}{Wdried\ sample}$$

Essential oil yield/ha was calculated from essential oil content and leaf biomass using the following formula:

$$Essential\ oil\ yield\ (kg/ha) = \frac{Essential\ oil\ Content\ \left(\frac{w}{w}\right)\ x\ Dry\ Leaf\ Yield\ (kg/ha)}{100}$$

Data analysis

To statistically analyse the differences in agro-morphological, EO content and yield of rose scented geranium caused bygrowing locations, five samples were taken from middle rows of each plot. Experimental data was statistically analysed by analysis of variance (ANOVA) using SAS v. 9.4 (SAS Institute Inc., Cary, North Carolina, USA) PROC GLM (2012) at $P < 0.05$. Person's correlation coefficient was employed to assess the association between different characters. Differences between means were assessed using the Least Significance Difference (LSD) test at $P < 0.05$.

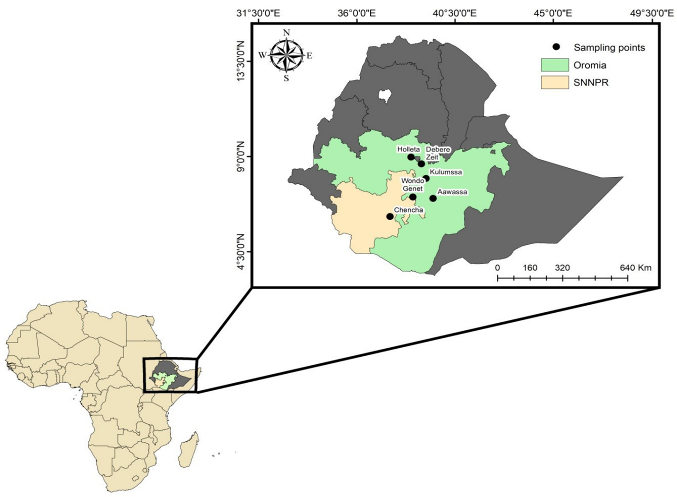

Figure 1: Map of the study areas along with their geographical coordinates.

Table 1: Summary of site descriptions used for adaptation testing of rose scented geranium for yield and yield component traits.

Testing locations	Altitude (m.s.s.l)	Latitude	Longitude	Soil pH	Soil type	Rain fall (mm)	Annual average temperature (°C)	
							Minimum	Maximum
Aawassa	1652	7°05' N	39°29' E	7.2	Sandy loam (Andosol)	964	12.94	27.34
Wondo Genet	1876	7°12' N	38°38' E	6.9	Sandy clay loam (Nitosol)	1000	12.02	26.72
Deberezeit	1891	8°44' N	38°58' E	6.6	Black heavy clay (Vertisol)	851	12.22	25.72
Kulumssa	2200	8°02' N	39°10' E	6.0	Clay loam soil (Gleysoil)	840	10.00	22.00
Holleta	2390	9°03' N	38°30' E	5.5	Red brown clay loam soil (Nitosol)	1100	06.13	22.20
Chencha	2618	6°13' N	37°34'E	4.9	Mollicnitisol	873	06.00	16.30

Results & Discussion

Influence of growing locations on the growth and yield performances of Rose-scented geranium

Combined analysis of variance for morphological, economic and chemical characters of rose-scented geranium tested over six locations are set out in table 2. Location exerted a very highly significant influence (*P* < 0.001) on the plant height, number of branches/plant, number of leaves/plant, fresh leaf yield/plant, leaf to stem ratio, internodes number/plant, fresh leaf yield/ha, EO content and EO yield of rose scented geranium. This indicates that these traits were influenced by a change in the environment. The significance of location effect was expected because Chencha, Hawassa, Wondo Genet, Kulmsa, Debrezeit, and Holletatypically vary in their altitude, soil pH, soil type, rainfall, and temperature (Table 1). In agreement with the results of the present study, Fehr (1991) [27] reported that every factor that is a part of the environment of a plant has the potential to cause differential performance. Likewise, Frankel et al. (1994) [28] and IRRI (1996) [29] reported that fluctuating features of the location such as rainfall, relative humidity, temperature, etc are some of the environmental factors that cause performance variation in plants. Even the results of the present correlation supported that the performances of the agro-morphological and chemical traits of rose-scented geranium are strongly associated with temperature, altitude, and soil pH (Table 3). Similar observations were reported for other essential oil bearing herbs in Ethiopia [30-34].

To examine the impact of selected environmental variables such as altitude, temperature and soil pH on agro-morphological, plant yield parameters and EO content, we conducted correlation analysis (Table 3). The correlation analysis revealed that altitude has a significant negative association with plant height (r=-0.99*), number of leaves per plant (r=-0.96*), number of internodes per plant (r=-0.97*), fresh leaf yield per plant (r=-0.94*), and fresh leaf yield per hectare (r=-0.91*); while a significant positive association was evident only with leaf to stem ratio (r=0.86*). As opposed to altitude, T° and soil pH have a significant positive association with plant height, number of leaves per plant, number of internodes per plant, fresh leaf yield per plant and fresh leaf yield per hectare. Also soil pH has a significant negative association with leaf to stem ratio (r=0.87*).

Previous studies have evidently shown that altitude, mean daily temperature and mean monthly evaporation have a considerable influence on phytochemical traits of aromatic plants and play a significant role in the quality and quantity EO production [35]. Besides, altitude has been proved to significantly affect the plant metabolism, chemical, pharmacological properties yield, composition and biology of plant extracts [36]. In addition, Etehadpour and Tavassolian (2019) [37] reported the important effect of ecological factors on EO yields and compositions; the most significant impact was reported of soil pH and latitude. These are not in accordance with the current study, which showed the non-significant influence of some ecological factors such as altitude, temperature and soil pH on EO content and EO yield/hectare (Table 3). In agreement with our study, Fernández-Sestelo and Carrillo (2020) [38] found little i.e. non-significant effect of altitude on EO yield and they interestingly could find the important effect of geographical location on EO production, which is in accordance with the achievement of the current study that proved a very highly significant impact of growing locations on all selected agro-morphological characters and EO production (Table 2). In general, the phytochemical diversity of medicinal and aromatic plants mostly varies through climatic and environmental conditions; some like soil texture and pH proved to have direct effect and some like geographical location had indirect influence on EO constituents and content [22].

Table 2: Combined Analysis of variance for morphological, economic and chemical characters of rose scented geranium tested over six locations.

Source of variation	df	Plant height	Branches number/plant	Leaves number/ plant	Leaf to stem ratio	Internodes number/plant	Fresh leaf yield/plant	Fresh leaf yield/ha	EO content	EO yield/ha
Replication	3	10.20	4.92	1232.43	0.029	189.72	562.38	401074	0.001	11.38
Location (L)	5	1340.3***	2167***	189445.40***	1.82***	44131.31***	272957.58***	178410411***	0.003***	222.89***
Error	15	9.14	10.54	964.38	0.03	51.13	369.26	710972	0.0003	15.40
CV (%)		5.49	8.42	9.05	10.66	7.43	3.87	3.39	23.95	23.95
$LSD_{0.05}$		3.08	3.30	31.59	0.19	7.27	19.54	857.74	0.02	0.02
R^2		97.09	98.73	98.55	95.32	99.76	99.45	99.67	83.20	83.20

***, very highly significant at P<0.001; **, highly significant at P <0.01by the least significant difference (LSD) test.

Table 3: Association of altitude, average annual temperature, and soil pH with morphological, economic and chemical characters of rose scented geranium.

	Plant height (cm)	Number of branches/ plant	Number of leaves/plant	Leaf to stem ratio	Number of internodes/plant	Fresh leaf yield/plant (kg)	Fresh leaf yield/ha (kg)	Essential oil content (%)	Essential oil yield/ ha (kg)
Altitude	-0.99***	-0.80ns	-0.96**	0.86*	-0.97**	-0.94**	-0.91*	-0.06ns	-0.69ns
Temperature	0.96**	0.63ns	0.87*	-0.79ns	0.99***	0.84*	0.86*	0.03ns	0.64ns
Soil pH	0.96**	0.78ns	0.97**	-0.87*	0.97**	0.95**	0.94**	0.06ns	0.67ns

***, very highly significant association ($P < 0.001$); **, a highly significant association ($P < 0.01$); *, significant association ($P < 0.05$); ns, non-significant association ($P > 0.05$) according to the Pearson's correlation coefficient test.

Performance of agro-morphological characters

The mean performances of agro-morphological characters of rose-scented geranium are summarized in table 4. The overall mean performance of plant height, number of branches/plant, number of leaves/plant, leaf to stem ratio and number of internodes/plant was 55.04 cm, 39, 343, 1.73 and 96, respectively. The mean performance of agro-morphological characters across the testing locations varied from 43.51 to 75.27 cm for plant height, 26 to 71 for number of branches/plant and 184 to 573 for number of leaves/plant, 1.38 to 2.37 for leaf to stem ratio and 18 to 219 for number of internodes/plant. The highest value for plant height, number of branches/plant, number of leaves/plant and number of internodes/plant was recorded at Hawassa and the highest value for leaf to stem ratio was recorded at Holleta and Chencha. The lowest values for plant height, number of branches/plant, number of leaves/plant were recorded at Chencha and Holleta. In contrast to our result, [39,8, 40-42] reported a shorter plant height range (45.9–64.1 cm) and lower number of branches/plant (8-27.6). The values obtained in our study are within the range of leaf to stem ratio values between 1.4 and 3.23 reported by Singh et al. (2011b) and Verma et al. (2011) [42,43]. The leaf number/plant obtained in our study is comparable with the result of Eiasu et al. (2017) [8] who showed a range of leaf number/plant value between 269.1 and 320.4 for rose-geranium plants treated with different levels of paclobutrazol and grown in the greenhouse. Contrastingly, lower values of leaf number/plant (131.5-171.8) and internode number per plant (68.12-75.37) were reported by Gebremeskel (2015) [39] tested under different plant spacing treatments in Ethiopia.

In the previous studies by Allard (1960), Poehlman and Sleper (1995) and Woliso and Kassahun (2020) [44-46], it has been described that the occurrence of performance variation in any plant is either due to hereditary differences in the plants, difference in the environments in which the plants are grown, or a combination of both. The correlation analysis also revealed that, compared with higher altitudes (with low soil pH and T°), lower altitudes (higher soil pH and T°) are favourable for the growth and development of the different vegetative characters except leaf to stem ratio (Table 3). Notably, as confirmed by the correlation analysis, leaf to stem ratio is inversely related with the other agro-morphological characters and was found higher in higher altitudes, which is characterized with low soil pH and annual average T°. Thus, leaf to stem ratio can be considered as an important character for determining the suitability of a given location for the cultivation of rose-scented geranium rather than assessing several agro-morphological factors. The temperature surrounding the plant is one of the key factors governing the rate of plant growth and development, and each plant requires optimum, minimum, and maximum temperature ranges (Hatfield JL and Prueger 2015) [47]. Thus, identification of suitable growing locations through well planned assessment activity for displaying the optimum production condition are the pre-requisite steps for effective production and sustainable build-up of the rose-scented geranium industry.

Table 4: Mean performance of morphological characters of rose scented geranium tested over six locations.

Locations	Plant height (cm)	Number of branches/plant	Number of leaves/plant	Leaf to stem ratio	Number of internodes/plant
Hawassa	75.27^{a}	70.67^{a}	573.08^{a}	1.38^{b}	219.27^{a}
Wondo Genet	63.45^{c}	37.97^{b}	481.20^{b}	1.45^{b}	203.05^{b}
Debrezeit	67.42^{b}	35.72^{bc}	383.18^{c}	1.41^{b}	193.07^{b}
Kulmsa	58.50^{d}	34.07^{c}	324.80^{d}	1.44^{b}	122.50^{c}
Holleta	47.07^{e}	26.37^{d}	211.45^{e}	2.37^{a}	117.72^{d}
Chencha	43.51^{f}	26.47^{d}	184.40^{e}	2.31^{a}	21.85^{e}
Over all mean	55.04	38.54	343.02	1.73	96.24
CV (%)	5.49	8.42	9.05	10.66	7.43
$LSD_{0.05}$	3.08	3.30	31.59	0.19	7.27

Mean followed by the same letter with in the same column are statistically non-significant at $P < 0.05$ according to the least significant difference (LSD) test.

Performanceof economic traits and chemical characters

The performance of rose-scented geranium for fresh leaf yield/plant, fresh leaf yield/ha, EO content (%) and EO yield/ha are summarized in table 5. The overall average fresh leaf yield/plant was 495.90 g. The highest value (740.89 g) was recorded at Hawassa followed by Wondo genet (609.60 g); the least value was recorded at Chencha (264.95 g). Contrastingly, a relatively lower range of fresh leaf yield/plant (196.2 to 282.5 g) was reported by Misra and Srivastava (2010) [48] under glasshouse condition. The higher fresh leaf yield/plant at Hawassa and Wondo Genet may be due to the development of better vegetative characters compared with Chencha testing locations (Table 4).

As shown in table 3, the correlation analysis revealed that the development of most of the studied vegetative characters was disfavoured with the growing conditions of Chencha (high altitude, low T°, and lower soil pH) as compared to Hawassa and Wondo Genet (lower altitude, high T°, and higher soil pH). Thus, the high fresh leaf yield/plant obtained at lower altitudes might be due the development of taller plants with more number of leaves/plant. As clearly shown in the correlation assessment result (Table 5), leaf yield/plant had a highly positive and significant association with plant height (r=0.91**) and number of leaves/plant (0.93**). The maximum values obtained for fresh leaf yield/plant in the present study is higher than the reports of Eiasu et al. (2017) and Gebremeskel (2015) [8,39] who showed a maximum value that lies between 92.26 and 450 g/plant under different spacing and paclobutrazol treatment in the greenhouse, indicating the merits of identifying suitable agro-ecologies for the cultivation of rose-scented geranium.

The overall average value of 24.88 t was obtained for fresh leaf yield/ha for rose-scented geranium tested over six locations. The highest (29.51 t) fresh leaf yield/ha was obtained at Hawassa and the lowest (18.52 t) at Chencha. In agreement with the present study, a herb yield range between 20.3 and 31.9 t/ha was reported for rose-scented geranium in India by Singh et al. (2011b) [42]. Kassahun et al. (2012) [49] also reported a wide range of leaf yield/ha form 13.7 to 34.3 t/ha under different plant population densities in Ethiopia.

The overall average EO content of 0.071% was obtained in this study. EO content varied from 0.052% for Holleta to 0.11 % for Kulmsa. In line with the present study, EO content range between 0.06 and 0.08% was reported by Motsa (2006) [15] in Republic of South Africa and 0.05 to 0.06% by Kassahun et al. (2012) [49] in Ethiopia. A relatively higher EO content range from 0.15 to 0.21% was reported by Misra et al. (2010) [40] through the supplementation of Cr and manipulation of reactive oxygen speies in greenhouse condition. Overall the test locations, an average EO yield of 15.75 kg was obtained. The average annual EO yield of rose-scented geranium was ranged from 10.01 to 21.85 Kg. The highest value was obtained at Kulmsa and the lowest at Chencha. EO yield range from 26.75 to 28.3 k/ha was reported by Motsa (2006) [15] in South Africa, 12.1 to 38 kg/ha was reported by Rao (2002) in India under different row spacing and cropping systems.

Table 5: Association of morphological and chemical characters of rose scented geranium.

	PH	NBPPL	NLPPL	LSR	NIPPL	FLYPPL	FLYPH	EOC	EOYPHA
PH	1	0.83*	0.95**	-0.90**	0.91**	0.96**	0.91**	0.19^{ns}	0.79^{ns}
NBPPL		1	0.87*	-0.60^{ns}	0.68^{ns}	0.84*	0.69^{ns}	-0.09^{n}s	0.48^{ns}
NLPPL			1	-0.84*	0.90**	0.97**	0.93**	0.01^{ns}	0.63^{ns}
LSR				1	-0.79^{ns}	-0.92**	-0.95**	-0.52^{ns}	-0.92**
NIPPL					1	0.86*	0.87*	0.01^{ns}	0.64^{ns}
FLYPPL						1	0.97**	0.24^{ns}	0.79^{ns}
FLYPH							1	0.53^{ns}	0.05^{ns}
EOC								1	0.75^{ns}
EOYPH									1

PH, plant height (cm); NBPPL, number of branches/plant; NLPPL, number of leaves/plant; LSR, leaf to stem ratio; NIPPL, number of internodes/plant; FLYPPL, fresh leaf yield/plant (kg); FLYPH, fresh leaf yield/ha (kg); EOC, essential oil content (%), EOYPHA, essential oil yield/ha (kg).

***, very highly significant association ($P < 0.001$); **, a highly significant association ($P < 0.01$); *, significant association ($P < 0.05$); ns, non-significant association ($P > 0.05$) according to Pearson's correlation coefficient test.

Conclusion

As agro-morphological yield and chemical traits obtained from the present study examined in different agro-ecologies of Ethiopia with various ecological parameters are comparable to different reports, it can generally be mentioned that rose scented geranium is found well adapted in Ethiopia. Therefore, it is possible to cultivate rose scented geranium both for herbal and EO production in mid highland to highland parts of the country. Notably, this comparable yield was obtained under open field conditions without the supplementation of any nutrients, and several other additional cultural practices. Thus, the possibility of enhancing the herbage yield, EO content, and EO yield of rose-scented geranium is very great under Ethiopian condition.

Acknowledgment

We would like to forward our sincere acknowledgment to Aromatic and Medicinal Plants Research Program for providing all the necessary facilities and support during the entire experimentation. Our acknowledgement also goes to all who made input for this study.

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